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(54) Title: POLYNUCLEOTIDE AND POLYPEPTIDE SEQUENCES ASSOCIATED WITH CNS DEPRESSANT SENSITIVITY AND METHODS OF USE THEREOF		
(57) Abstract The invention provides polynucleotide and polypeptide sequences associated with CNS depressant sensitivity, and methods for identifying and classifying candidate CNS depressants as well as methods to identify agents which may modulate CNS depressant action. The invention also provides mouse mGluR5 polynucleotide and polypeptide sequences.		

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**POLYNUCLEOTIDE AND POLYPEPTIDE SEQUENCES ASSOCIATED WITH
CNS DEPRESSANT SENSITIVITY AND METHODS OF USE THEREOF**

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims the priority benefit of U.S. Provisional Patent Application No. 60/073,190, filed January 30, 1998. The priority application is hereby incorporated herein by reference in its entirety.

STATEMENT OF RIGHTS TO INVENTIONS MADE UNDER
10 FEDERALLY SPONSORED RESEARCH

 This invention was made in part during work supported by grants from the National Institutes of Health (NIH) NIAAA RO1-AA08940 and NIGMS RO1-GM55635. The government has certain rights in this invention.

15 TECHNICAL FIELD

 This invention relates to polynucleotide and polypeptide sequences associated with CNS depressant sensitivity, as well as methods using these sequences.

BACKGROUND ART

20 Central nervous system (CNS) depressants have been used for a variety of purposes, including anesthesia. CNS depressants are also socially, economically and medically significant in terms of drug use and abuse, such as ethanol, barbituate, and sedative consumption. While several useful anesthetics have been developed, such as propofol, improved anesthetics are still needed. Furthermore, the basis of propensity toward excess
25 ethanol consumption, or differential sensitivity toward this and other CNS depressants, is still not known.

 Several animal models have been used to study the basis of CNS depressant action. Simpson and Johnson (1996) *Int. Rev. Neurobiol.* 39:223-241. LS (long sleep) and SS (short sleep) mice were initially selected on the basis of differential ethanol sensitivity.
30 Simpson and Johnson (1996); McClearn and Kakihana (1981) "Development of Animal Models as Pharmacogenic Tools", McClearn et al, eds, Research Monograph No. 6 at 147-159 (U.S. Dept. Health Human Services). In addition to ethanol, the LS and SS mice are

differentially sensitive to a variety of other agents having properties of CNS depressants. Simpson et al. (1996) *Anesth. Analg.* 82:1-5; Markel et al. (1996) *Mam. Genome* 7:408-412; Simpson and Johnson (1996). These CNS depressants include chloral hydrate, enflurane, etomidate, flurazepam, halogenated ethanols, isoflurane, ketamine, midazolam, paraldehyde, MK-801, urethane and propofol. These agents have diverse chemical structures suggesting that the LS and SS mice have been selected, at least in part, for genes that influence sensitivity to multiple classes of CNS depressants. However, LS and SS mice do not differ in sensitivity to ether or halothane, (Baker et al. (1980) *Pharmacol. Biochem. and Behav.* 12:691-695), indicating that there has not been uniform selection for differential sensitivity to all anesthetic agents.

LS and SS mice exhibit significant CNS differences in their sensitivity to propofol-induced anesthesia. Simpson and Blednov (1996) *Anesth. Analg.* 82:327-331. Given the same 20 mg/kg dose via the retroorbital sinus, SS mice slept about 3.5 minutes (SEM = 0.4 min) and LS mice slept about 2.2 minutes longer, with no difference between males and females. Moreover, although propofol has a potent effect on GABA_A receptors, (Tanelian et al. (1993) *Anesthesiology* 78:757-776), LS and SS mice did not exhibit significant differences in GABA_A-activated chloride channels. However, the genetic locus contributing to this differential sensitivity has not been discovered.

The metabotropic glutamate receptors (mGluRs) are G protein-coupled receptors capable of activating a variety of intracellular second messenger systems following the binding of glutamate or other potent agonists including quisqualate and 1-aminocyclopentane-1,3-dicarboxylic acid (*trans*-ACPD). Schoepp et al. (1990) *Trends Pharmacol. Sci.* 11:508; Schoepp and Conn (1993) *Trends Pharmacol. Sci.* 14:13.

Activation of different metabotropic glutamate receptor subtypes *in situ* elicits one or more of the following responses: activation of phospholipase C, increases in phosphoinositide (PI) hydrolysis, intracellular calcium release, activation of phospholipase D, activation or inhibition of adenylyl cyclase, increases and decreases in the formation of cyclic adenosine monophosphate (cAMP), activation of guanylyl cyclase, increases in the formation of cyclic guanosine monophosphate (cGMP), activation of phospholipase A₂, increases in arachidonic acid release, and increases or decreases in the activity of voltage- and ligand-gated ion channels (Schoepp and Conn (1993); Schoepp (1994) *Neurochem. Int.* 24:439; Pin and Duvoisin (1995) *Neuropharmacology* 34:1).

Thus far, eight distinct mGluR subtypes have been isolated via molecular cloning, and named mGluR1 to mGluR8 according to the order in which they were discovered. See, for example, Nakanishi (1994) *Neuron* 13:1031; Pin and Duvoisin (1995) *Neuropharmacology* 34:1; Knopfel et al. (1995) *J. Med. Chem.* 38:1417. Further diversity occurs through the expression of alternatively spliced forms of certain mGluR subtypes. Pin et al. (1992) *Proc. Natl. Acad. Sci.* 89:10331; Minakami et al. (1994) *BBRC* 199:1136. All of the mGluRs appear to be structurally similar, in that they are proposed to be single subunit membrane proteins possessing a large amino-terminal extracellular domain (ECD) followed by seven putative transmembrane domain (7TMD) comprising seven putative membrane spanning helices connected by three intracellular and three extracellular loops, and an intracellular carboxy-terminal domain of variable length (cytoplasmic tail) (CT). The eight mGluR subtypes have unique patterns of expression within the mammalian CNS that in many instances are overlapping. Masu et al. (1991) *Nature* 349:760; Martin et al. (1992) *Neuron* 9:259; Ohishi et al. (1993) *Neurosci.* 53:1009; Tanabe et al. (1993) *J. Neurosci.* 13:1372; Ohishi et al. (1994) *Neuron* 13:55; Abe et al. (1992) *J. Biol. Chem.* 267:13361; Nakajima et al. (1993) *J. Biol. Chem.* 267:13361; Nakajima et al. (1993) *J. Biol. Chem.* 268:11868; Okamoto et al. (1994) *J. Biol. Chem.* 269:1231; Duvoisin et al. (1995) *J. Neurosci.* 15:3075.

The eight mGluRs have been subdivided into three groups based on amino acid sequence identities, the second messenger systems they utilize, and pharmacological characteristics. Nakanishi (1994) *Neuron* 13:1031; Pine and Duvoisin (1995) *Neuropharmacology* 34:1; Knopfel et al. (1995) *J. Med. Chem.* 38:1417.

The Group I mGluRs comprise mGluR1, mGluR5 and their alternatively spliced variants. The binding of agonists to these receptors results in the activation of phospholipase C and the subsequent mobilization of intracellular calcium. For example, *Xenopus* oocytes expressing recombinant mGluR1 receptors have been utilized to demonstrate this effect indirectly by electrophysiological means. Masu et al. (1991); Pin et al. (1992). Similar results were achieved with oocytes expressing recombinant mGluR5 receptors. Abe et al. (1992); Minakami et al. (1994). Alternatively, agonist activation of recombinant mGluR1 receptors expressed in Chinese hamster ovary (CHO) cells stimulated PI hydrolysis, cAMP formation, and arachidonic acid release as measured by standard biochemical assays. Aramori and Nakanishi (1992) *Neuron* 8:757. In comparison,

activation of mGluR5 receptors expressed in CHO cells stimulated PI hydrolysis and subsequent intracellular calcium transients but no stimulation of cAMP formation or arachidonic acid release was observed. Abe et al (1991).

5 The Group II mGluRs include mGluR2 and mGluR3. Activation of these receptors as expressed in CHO cells inhibits adenylyl cyclase activity via the inhibitory G protein, G_i , in a pertussis toxin-sensitive fashion. Tanabe et al. (1992) *Neuron* 8:169; Tanabe et al. (1993).

10 The Group III mGluRs include mGluR4, mGluR6, mGluR7 and mGluR8. Like the Group II receptors these mGluRs are negatively coupled to adenylate cyclase to inhibit intracellular cAMP accumulation in a pertussis toxin-sensitive fashion when expressed in CHO cells (Tanabe et al. (1993); Nakajima et al. (1993); Okamoto et al. (1994); Duvoisin et al. (1995).

15 Several reports indicate that various mGluRs may be affected by various anesthetics. See, e.g., Dildy-Mayfield et al. (1996) *J. Pharmacol. Exp. Ther.* 276:1058-1065 (describing experiments addressing the effect of various anesthetics on certain mGluRs, but not mGluR5); Orser et al. (1995) *Br. J. Pharmacol.* 116:1761-1768 (describing experiments addressing inhibition by propofol of N-methyl-D-aspartate subtype of glutamate receptor); Miyamoto et al. (1994) *Eur. J. Pharmacol.* 260:99-102 (describing experiments addressing delay in recovery from halothane anesthesia by agonists for
20 metabotropic glutamate receptors).

Nucleic acids encoding human metabotropic glutamate receptors mGluR1, mGluR2, mGluR3 and mGluR5 are described in U.S. Pat. 5,521,297. See also U.S. Pat. 5,202,257. Human mGluR5 is cloned and characterized by Minakami et al. (1994) *Biochem. Biophys. Res. Comm.* 199:1136-1143. Rat mGluRs polynucleotide and
25 polypeptide sequences are described in Abe et al. (1992). See also Minakami et al. (1993) *Biochem. Biophys. Res. Comm.* 194:622-627. The 550 base nucleotide sequence containing a single exon (of about 100 nucleotides) of mouse mGluR5 has been reported. Minakami et al. (1995) *J. Neurochem.* 65:1536-1542.

30 There is a continuing need to develop new CNS depressants, particularly anesthetics, which exert fewer side effects. A defined molecular target may allow the design of anesthetics which act specifically at neural tissue, thus avoiding side effects related to non-specific actions at other organ systems. There is also a need to refine and/or

modulate the action of anesthetics, particularly in certain individuals, such as at-risk individuals.

All references cited herein are incorporated by reference in their entirety.

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DISCLOSURE OF THE INVENTION

The present invention provides methods using polynucleotide and polypeptide sequences associated with CNS sensitivity, as well as the polynucleotide and polypeptide sequences themselves. In particular, these methods are used to identify, inter alia, agents which may exhibit CNS depressant activity and/or modulate CNS depressant activity.

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Accordingly, in one aspect, the invention provides a method for identifying an agent which may exhibit CNS depressant activity, comprising the steps of: (a) introducing a polynucleotide comprising a mouse polynucleotide sequence associated with CNS depressant sensitivity into a suitable host cell, wherein the mouse polynucleotide sequence corresponds to a polynucleotide sequence of yeast artificial chromosome YRT2; (b) contacting host cell of step (a) with at least one agent to be tested; and (c) analyzing at least one characteristic associated with expression of the mouse YRT2 polynucleotide, wherein an agent is identified by its ability to modulate expression of the mouse YRT2 polynucleotide.

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In another aspect, the invention provides a method of classifying a CNS depressant, comprising the steps of: (a) introducing a polynucleotide comprising a mouse polynucleotide sequence associated with CNS depressant sensitivity into a suitable host cell, wherein the mouse polynucleotide sequence corresponds to a polynucleotide sequence of yeast artificial chromosome YRT2; (b) contacting host cell of step (a) with at least one agent to be tested; (c) analyzing at least one characteristic associated with expression of the mouse YRT2 polynucleotide, wherein an agent is identified by its ability to modulate expression of the mouse YRT2 polynucleotide, and wherein a CNS depressant which modulates expression may fall within a class of CNS depressants which displays differential reactivity in LS and SS mice.

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In another aspect, the invention provides a method of identifying an agent which may modulate CNS depressant sensitivity in an individual, said method comprising: (a) introducing a polynucleotide comprising a mouse polynucleotide sequence associated with CNS depressant sensitivity into a suitable host cell, wherein the mouse polynucleotide

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sequence corresponds to a polynucleotide sequence of yeast artificial chromosome YRT2;
(b) contacting host cell of (a) with at least one agent to be tested; and (c) analyzing at least
one characteristic associated with expression of the mouse YRT2 polynucleotide, wherein
an agent is identified by its ability to modulate expression of the mouse YRT2
5 polynucleotide.

In another aspect, the invention provides a method of identifying an agent which
may exhibit CNS depressant activity, comprising the steps of: (a) introducing a
polynucleotide comprising a metabotropic glutamate receptor polynucleotide sequence into
a suitable host cell; (b) contacting host cell of step (a) with at least one agent to be tested;
10 (c) analyze at least one characteristic associated with expression of the metabotropic
glutamate receptor polynucleotide, wherein an agent is identified by its ability to modulate
expression of the mammalian metabotropic glutamate receptor polynucleotide. The
metabotropic glutamate receptor may be mammalian.

In another aspect, the invention provides a method of classifying a CNS depressant,
15 comprising the steps of: (a) introducing a polynucleotide comprising a mammalian
metabotropic glutamate receptor polynucleotide sequence into a suitable host cell; (b)
contacting host cell of step (a) with at least one agent to be tested; (c) analyzing at least one
characteristic associated with expression of the metabotropic glutamate receptor
polynucleotide, wherein an agent is identified by its ability to modulate expression of the
20 metabotropic glutamate receptor polynucleotide sequence, wherein a CNS depressant
which modulates the metabotropic glutamate receptor polynucleotide expression may fall
within a class of CNS depressants which displays differential reactivity in LS and SS mice.

In another aspect, the invention provides an isolated polynucleotide comprising a
polynucleotide encoding a mouse mGluR5 polypeptide, wherein the mouse mGluR5
25 polypeptide is at least 10 contiguous amino acids of SEQ ID NO:2 and exhibits mGluR5
activity, and wherein the at least 10 contiguous amino acids are not depicted in SEQ ID
NO:6 or SEQ ID NO:7.

In another aspect, the invention provides an isolated polynucleotide comprising a
polynucleotide of at least about 25 contiguous nucleotides of SEQ ID NO:1, wherein the at
30 least about 25 contiguous nucleotides are not depicted in SEQ ID NO:3 or SEQ ID NO:5.

In another aspect, the invention provides a polynucleotide comprising a region of at
least 25 contiguous nucleic acids of SEQ ID NO:1, said region having at least about 97%

sequence identity to a sequence in SEQ ID NO:1. The region is not depicted in SEQ NO:3 or SEQ ID NO:5.

In another aspect, the invention provides an isolated polypeptide comprising at least about 5 contiguous amino acids of the sequence of SEQ ID NO:2, wherein the isolated polypeptide exhibits an mGluR5 function, and wherein the about 5 contiguous amino acids are not depicted in SEQ ID NO:6 or SEQ ID NO:7.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a dose-response curve depicting propofol dose (milligrams per kilogram) versus sleep time (minutes) in Long Sleep (LS; open triangle) and Short Sleep (SS; solid triangle) mouse lines. Sleep time is defined as the duration of loss of righting reflex. Each point represents the mean sleep time \pm SEM for $n = 6-10$. For every dose tested, the LS and SS lines are differentially sensitive ($P < 0.01$).

Fig. 2 is a bar graph depicting sleep times (minutes) in LS (open bars) and SS (hatched bars) mice. Routes of administration were jugular cannula (JUG; left bar of each pair) or retroorbital sinus (ROS; right bar of each pair) injections. Each bar represents means sleep time \pm SEM ($n = 7-9$). There was a small, statistically significant difference in Short Sleep (SS) sleep times between the two techniques ($P < 0.02$) but no difference in Long Sleep (LS) sleep times ($P = 0.09$).

Fig. 3 is a bar graph depicting effects of propofol on muscimol-stimulated $^{36}\text{Cl}^-$ uptake in Long Sleep (LS, open bars) and Short Sleep (SS, hatched bars) brain membranes. Bars express the mean \pm SEM. Eight to 10 female LS or SS mice were used per group.

Figs. 4 (A) and (B) are graphs depicting duration of LORR (loss of righting reflex) and BLA (brain levels of propofol at awakening) in pigmented and albino LSXSS RI strains. Pigmented strains are shown as black bars; albino RI strains are shown as white bars. Error bars indicate standard error of the mean. In Fig. 4 (B), the strain distribution pattern mean LORR for propofol for the pigmented RI strains is indicated by the filled ovals, and the means for the albino RI strains is indicated by the open ovals. The numbers next to the ovals indicate the RI strain(s) having that mean. RI strain 32 had a mean LORR of 4.4 minutes (SEM = 0.7) for females and 6.6 minutes (SEM = 1.1) for males, but it was not included in this analysis because at that time it was still segregating for the albino mutation.

Fig. 5 depicts a genetic map of murine chromosome 7 showing LOD scores for positioning *Lorpl* using the LSXSS RIs. Markers include the albino mutation (*c*), the *pink-eyed dilution* mutation (*p*) and six *D7Mit* SSLPs. The dashed line indicates the recommended threshold for statistical significance. Data were combined for males and females.

Fig. 6 depicts a genetic map of murine chromosome 7 showing confirmation of *Lorpl* from F_2 intercrosses between ILS and ISS mice. Markers are *D7Mit* SSLPs. Marker *D7Mit31* is within the *Tyr* gene near the site of albino mutation, *c*. The dashed line indicates the recommended threshold for statistical significance. Data were combined for males and females ($N = 164$).

Figs. 7 (A) and (B) are bar graphs depicting propofol LORRs of (ISS *c/c* \times C57BL/6 *c^{2j}/C*) F_1 s and of ILS and ISS mice with tyrosine. ILS (black bars) and ISS (white bars) mice coinjected with tyrosine (indicated as pr + tyr), substrate of the tyrosinase enzyme encoded by the albino gene, also did not exhibit significant differences in LORR ($p = 0.78$, ANOVA two-tailed significance) from controls at a concentration of tyrosine (200 mg/kg) previously shown to differentially alter ethanol-induced LORRs in LS and SS. "n" indicates the number of mice (males and females) tested. Error bars indicate standard error of the mean.

Fig. 8 depicts a genetic map of murine chromosome 7 showing LOD scores for positioning *Lorpl* using the LSXSS RIs. Results are shown for propofol, isoflurane, enflurane, and ethanol. The left-hand panels show data for females; the right-hand panels show data for males.

Fig. 9 depicts a genetic map of murine chromosome 7 showing LOD scores for positioning *Lorpl* using LSXSS RIs. This experiment was performed using etomidate.

Fig. 10 depicts a genetic map of murine chromosome 7 showing confirmation of *Lorpl* from F_2 intercrosses between ILS and ISS mice. This experiment was performed using etomidate.

Fig. 11 is a schematic depiction of YAC YRT2, containing a 250 kb genomic insert containing the mouse tyrosinase and mGluR5 genes. Expanded depiction of vector arms are shown below. Markers and functional elements from the vector arms are shown as filled arrows (except the open circle, which indicates the centromere). Numbers 1-5 indicate the exons of the tyrosinase gene. Tel, telomeres; Amp, ampicillin resistance gene;

Tk, thymidine kinase gene of herpes simplex virus; Cen, centromere (*CEN4*); associated with the Gal-1 promoter (*GAL1*); *TRP1* and *URA3*, yeast markers.

Fig. 12 is a bar graph comparing sleep times of SS (non-transgenic; left bar of each pair) and transgenic mice containing YRT2 (right bar of each pair) when given propofol. N = number of injections.

Fig. 13 is a schematic showing the strategy for analyzing mouse DNA sequences present on YRT2.

Fig. 14 (A)-(G) depicts the nucleotide sequence and a conceptual amino acid translation of most of the mouse mGluR5 coding region (SEQ ID NOS:1 and 2).

Fig. 15 shows a portion of mouse mGluR5 nucleotide sequence obtained from the Genbank database and its conceptual amino acid translation (SEQ ID NOS:3 and 4).

Fig. 16 (A)-(G) depicts the nucleotide and amino acid sequences of rat mGluR5 (SEQ ID NOS:5 and 6).

Fig. 17 (A)-(C) depicts the amino acid sequence of human mGluR5 (SEQ ID NO:7).

MODES FOR CARRYING OUT THE INVENTION

We have discovered mouse polynucleotide sequences that are associated with a particular, significant, biological phenomenon, namely sensitivity to CNS depressants. In particular, the sensitivity referred to herein (which may also be considered as decreased resistance) is with respect to a particular class of CNS depressants defined by the differential reactivity of LS and SS mice to these CNS depressants. This class of CNS depressants includes, but is not limited to, ethanol, chloral hydrate, enflurane, etomidate, flurazepam, halogenated ethanols, isoflurane, ketamine, midazolam, paraldehyde, MK-801, urethane and propofol. This discovery makes possible useful screening assays for agents which may exhibit CNS depressant activity as well as agents which may modulate CNS depressant sensitivity. This discovery may also lead to finding corresponding human sequences which confer differential sensitivity to CNS depressants, including ethanol, which in turn could provide the basis for powerful diagnostics in the context of, for example, ethanol sensitivity or vulnerability to ethanol addiction as well as determination of presence of risk factors for anesthesia administration.

Mouse polynucleotide sequences were identified based on association with a particular biological function, namely, resistance to CNS depressant activity. Briefly, the polynucleotide and polypeptide sequences that form the basis of the screening methods described herein were discovered and obtained by: (a) mapping the genetic locus
5 associated with the phenomenon of differential CNS depressant response; (b) complementation of the SS (resistant) phenotype to an LS (sensitive) phenotype by a YAC denoted YRT2 containing a 250 kb mouse genomic insert containing a tightly linked locus to the observed differential response phenomenon, namely, the *albino* (*tyr*) locus encoding tyrosinase. Thus, sequences contained in this YAC are associated with differential CNS
10 depressant response. A schematic of YRT2 is depicted in Fig. 11.

The mapping experiments were conducted with propofol, enflurane, isoflurane, ethanol, and etomidate. In all cases, the trait associated with the differential response (the *Lorpl* locus) was significantly linked to the murine tyrosinase (*albino*) locus on chromosome 7. Thus, the sequences associated this differential sensitivity likely
15 encompass the entire class of CNS depressants described above.

Definitions

As used herein, a "YRT2 polynucleotide" is a mouse polynucleotide sequence that is contained in, or corresponds to, a polynucleotide sequence within the yeast artificial
20 chromosome (YAC) vector denoted YRT2, which is described and discussed herein. A schematic depiction of YRT2 is provided in Fig. 11. As the mouse polynucleotide sequences reflect a genomic configuration (i.e., the insert was obtained from a mouse genomic library), and the identity of at least part of the YAC insert is known, as well as the insert's genomic location, it is clear to those skilled in the art that these sequences may be
25 obtained from sources other than YRT2 itself. It is understood that the definition of "YRT2 polynucleotide" includes, but is not limited to, polynucleotides contained within the tyrosinase gene and/or the mGluR5 gene. A gene, as understood by those of skill in the art, contains coding as well as non-coding sequences (such as 5' and 3' flanking sequences and introns). Accordingly, any definitions pertaining to YRT2 polynucleotides also apply to
30 tyrosinase and mGluR5 polynucleotides.

A "YRT polypeptide" is a polypeptide that is encoded by a YRT2 polynucleotide. It is understood that the definition of "YRT2 polypeptide" includes, but is not limited to,

polypeptides encoded within the tyrosinase gene and/or the mGluR5 gene. Accordingly, any definitions pertaining to YRT2 polypeptides also apply to tyrosinase and mGluR5 polypeptides.

5 A sequence "corresponding" to a sequence in YRT2 means that, when compared (i.e., aligned), the sequences share at least about 80% sequence identity, preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, even more preferably at least about 95% sequence identity, even more preferably at least about 98% sequence identity, most preferably 100% sequence identity. As discussed below, the term "corresponding" is used to emphasize that the sequences used in the methods
10 described in this invention need not literally have been obtained from YRT2, but need only correspond in terms of contiguous nucleic acid composition to the sequences of YRT2. It is also understood that a sequence corresponding to YRT2 also applies to any polypeptide sequences encoded in the mouse polynucleotide sequence of YRT2, and that, as noted above for polynucleotide sequence identity, need not be a perfect correspondence to the
15 sequence of YRT2 and need not be obtained by expressing YRT2 sequences per se.

As used herein, "expression" encompasses any level leading to, involving, and/or resulting from production of a protein product. Accordingly, expression includes any aspect of transcription (including the modulation or effect of control sequences such as enhancers or promoters), translation, processing, sorting, post-translational modification(s),
20 folding, conformation, assembly, subcellular and/or extracellular localization and/or position, binding, and/or effector function(s) (such as those involved in a signal transduction, or second messenger, pathway)...

A characteristic which is associated with "modulation" of expression is a characteristic which is associated with an alteration, increase or decrease, in expression.

25 A "control region" of a gene is any sequence, of any length, which affects gene expression, most usually transcription. Examples of control regions include, but are not limited to, promoters and enhancers.

An "mGluR polynucleotide" refers to a polynucleotide contained within (or in) an mGluR gene. Examples of mGluR genes have been discussed and are known in the art.
30 The mGluR polynucleotide may be of any origin, preferably eukaryotic, more preferably mammalian, such as mouse, human, or rat. However, it is understood that, due to sequence homology, an mGluR polynucleotide may also be obtained from other cell types, such as C.

elegans, Drosophila, and yeast. For example, GenBank provides a homolog of mGluR as derived from *C. elegans*.

As used herein, "mGluR5 polynucleotide" refers to a polynucleotide contained within (or in) an mGluR5 gene. A "mouse mGluR5 polynucleotide" refers to a polynucleotide contained within (or in) a mouse mGluR5 gene. Similarly, a "mammalian mGluR5 polynucleotide" or "human mGluR5 polynucleotide" refers to a mammalian or human polynucleotide contained within or in a mammalian or human mGluR5 gene, respectively. A sequence of part of the mouse mGluR5 gene is depicted within SEQ ID NO:1. A "fragment" or "region" of mGluR5 gene is a portion of the mGluR5 gene, and as such may contain coding and/or non-coding sequences. For example, SEQ ID NO:1 contains a fragment of the mGluR5 depicting the coding sequence. Preferably, a fragment of mGluR5 comprises at least 10 contiguous nucleotides, more preferably at least 15, more preferably at least 25, more preferably at least 30, more preferably at least 50, more preferably at least 100, more preferably at least 150, more preferably at least 200, more preferably at least 250, more preferably at least 300 contiguous nucleotides.

"mGluR5" refers to a protein (polypeptide) product encoded in a mGluR5 gene. As noted for mGluR5 polypeptides above, a type (indicating genetic origin) of mGluR5 is specified, such as mouse, human, or mammalian. SEQ ID NO:2 depicts a conceptual translation of an open reading frame of mouse mGluR5. A "fragment" or "region" of mGluR5 (i.e., full-length) is a portion of the mGluR5 gene product. It is understood that mGluR5 may exist in more than one form, due to, for example, alternative splicing.

"mGluR5 function" or "mGluR5 activity" refers to an activity or characteristic associated with expression of mGluR5. These functions include, but are not limited to, (a) transcription; (b) translation, including post-translational processing; (c) regulation by other polypeptides; (d) binding ligand; (e) signal transduction pathway (i.e., second messenger system) activity. For mGluR5, these signal transduction pathway activities include, but are not limited to, PI hydrolysis and mobilization of intracellular calcium.

As used herein, a characteristic which is associated with a "modulation" of mGluR5 function or activity is a characteristic which is associated with an alteration, increase or decrease, in mGluR5 function or activity. As used herein, "modulation of mGluR5 activity" means any change in any measurable parameter along the functional pathway of mGluR5. The "functional pathway" of mGluR5 includes transcription, translation,

processing (including pre-and post-translational processing), positioning and/or conformation of receptor (either in solution or within a membrane context), binding to receptor, activities triggered by binding to the receptor (i.e., signal transduction pathway), and/or modulation of other neural receptor activities.

5 “Suitable reaction conditions” refer to those conditions which allow a characteristic to be measured to occur. For example, if the characteristic is binding, then suitable reaction conditions are those which allow this binding to occur. If the characteristic is a signal transduction activity, then suitable reaction conditions are those which allow this activity to occur. Depending on which characteristic will be measured, the reaction conditions will
10 vary. Determination of suitable reaction conditions is well within the skill of those in the art.

 A sequence, whether polynucleotide or polypeptide, “depicted in” a SEQ ID NO, means that the sequence is present as an identical contiguous sequence in the sequence of the SEQ ID NO. Conversely, a contiguous sequence that is “not depicted in” a SEQ ID NO
15 means that the contiguous sequence is not present as an identical contiguous sequence in the sequence of the SEQ ID NO. The term “contiguous” sequence and referring to, for example, an amino acid sequence of a specified length, are interchangeable in this context.

 As used herein, a “polynucleotide” is a polymeric form of nucleotides of any length, which contain deoxyribonucleotides, ribonucleotides, and/or their analogs. The terms
20 “polynucleotide” and “nucleotide” as used herein are used interchangeably. Polynucleotides may have any three-dimensional structure, and may perform any function, known or unknown. The term “polynucleotide” includes double-, single-stranded, and triple-helical molecules. Unless otherwise specified or required, any embodiment of the invention described herein that is a polynucleotide encompasses both the double-stranded
25 form and each of two complementary single-stranded forms known or predicted to make up the double stranded form. Not all linkages in a polynucleotide need be identical.

 The following are non-limiting examples of polynucleotides: a gene or gene fragment, exons, introns, mRNA, tRNA, rRNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any
30 sequence, isolated RNA of any sequence, nucleic acid probes, primers, and adaptors. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and

nucleotide analogs. The use of uracil as a substitute for thymine in a deoxyribonucleic acid is also considered an analogous form of pyrimidine.

5 In the context of polynucleotides, a "linear sequence" or a "sequence" is an order of nucleotides in a polynucleotide in a 5' to 3' direction in which residues that neighbor each other in the sequence are contiguous in the primary structure of the polynucleotide. A "partial sequence" is a linear sequence a portion of a polynucleotide, wherein the polynucleotide is known to comprise additional residues in one or both directions.

10 If present, modification to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. Other types of modifications included in this definition are, for example, "caps", substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, 15 phosphoamidates, cabamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with 20 modified linkages (e.g., α -anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotide(s).

25 Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid supports. The 5' and 3' terminal OH groups can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups.

30 Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, but not limited to, 2'-O-methyl-, 2'-O-allyl-, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, α -anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs and abasic nucleoside analogs such as methyl riboside.

Although conventional sugars and bases will be used in applying the method of the invention, substitution of analogous forms of sugars, purines and pyrimidines can be advantageous in designing a final product, as can alternative backbone structures like a polyamide backbone.

5 A polynucleotide or polynucleotide region has a certain percentage (for example, 75%, 80%, 85%, 90%, 95%, 98%, or 99%) of "sequence identity" to another sequence means that, when aligned, that percentage of bases are the same in comparing the two sequences. As is discussed further herein, "sequence identity" can be indicated by one of several measurable parameters, such as sequence alignment techniques (most typically
10 performed with the use of standard alignment programs) and hybridization techniques, both of which are discussed herein.

"Hybridization" refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson-Crick base pairing, Hoogsteen
15 binding, or in any other sequence-specific manner. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi-stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of a PCR, or the enzymatic cleavage of a polynucleotide by a ribozyme.

20 When hybridization occurs in an antiparallel configuration between two single-stranded polynucleotides, those polynucleotides are described as "complementary". A double-stranded polynucleotide can be "complementary" to another polynucleotide if hybridization can occur between one of the strands of the first polynucleotide and the second. Complementarity (the degree that one polynucleotide is complementary with another) is
25 quantifiable in terms of the proportion of bases in opposing strands that are expected to form hydrogen bonding with each other, according to generally accepted base-pairing rules.

A "primer" is a short polynucleotide, generally with a free 3' -OH group, that binds to a target potentially present in a sample of interest by hybridizing with the target, and thereafter promoting polymerization of a polynucleotide complementary to the target.

30 An "adaptor" is a short, partially-duplexed polynucleotide that has a blunt, double-stranded end and a protruding, single-stranded end. It can be ligated, through its double-stranded end, to the double-stranded end of another polynucleotide. This provides known

sequences at the ends of thus modified polynucleotides. Often adaptors contain specific sequences for primer binding and/or restriction endonuclease digestion.

5 A "probe" when used in the context of polynucleotide manipulation refers to a polynucleotide which is provided as a reagent to detect a target potentially present in a sample of interest by hybridizing with the target. Usually, a probe will comprise a label or a means by which a label can be attached, either before or subsequent to the hybridization reaction. Suitable labels include, but are not limited to radioisotopes, fluorochromes, chemiluminescent compounds, dyes, and enzymes.

10 "Transformation" or "transfection" refers to the insertion of an exogenous polynucleotide into a host cell, irrespective of the method used for the insertion, for example, lipofection, transduction, infection or electroporation. The exogenous polynucleotide may be maintained as a non-integrated vector, for example, a plasmid, or alternatively, may be integrated into the host cell genome.

15 A polynucleotide is said to "encode" a polypeptide if, in its native state or when manipulated by methods well known to those skilled in the art, it can be transcribed and/or translated to produce the polypeptide or a fragment thereof. For purposes of this invention, and to avoid cumbersome referrals to complementary strands, the anti-sense (or complementary) strand of such a polynucleotide is also said to encode the sequence; that is, a polynucleotide sequence that "encodes" a polypeptide includes both the conventional
20 coding strand and the complementary sequence (or strand).

The terms "polypeptide", "oligopeptide", "peptide" and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, it may be interrupted by non-amino acids, and it may be assembled into a complex of more than one polypeptide
25 chain. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for
30 example, unnatural amino acids, etc.), as well as other modifications known in the art.

In the context of polypeptides, a "linear sequence" or a "sequence" is an order of amino acids in a polypeptide in an N-terminal to C-terminal direction in which residues that

neighbor each other in the sequence are contiguous in the primary structure of the polypeptide. A "partial sequence" is a linear sequence of a portion of a polypeptide, wherein the polypeptide is known to comprise additional residues in one or both directions.

5 A polypeptide "fragment" (also called a "region") is a polypeptide comprising an amino acid sequence that has at least 5 contiguous amino acids of a sequence, more preferably at least 10 contiguous amino acids, more preferably at least about 15 contiguous amino acids, even more preferably at least about 25 contiguous amino acids, even more preferably at least about 30 contiguous amino acids, even more preferably at least about 40 contiguous amino acids, or even larger.

10 A polypeptide or polypeptide region (or fragment) has a certain percentage of "sequence identity" (for example, 75%, 80%, 85%, 90%, 95%, 98%, 99%) to another sequence means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. As discussed herein, those skilled in the art generally use commercially available alignment programs to determine degree of sequence identity.

15 A "fusion polypeptide" is a polypeptide comprising regions in a different position than occurs in nature. The regions may normally exist in separate proteins and are brought together in the fusion polypeptide, or they may normally exist in the same protein but are placed in a new arrangement in the fusion polypeptide.

20 A "functionally preserved" variant of a YRT2 polynucleotide or YRT2 polypeptide is a sequence which retains at least one aspect of YRT2 function. Functionally preserved variants can be due to differences in linear sequence, arising from, for example, single base mutation(s), addition(s), deletion(s), and/or modification(s) of the bases. The difference can also arise from changes in the sugar(s) and/or linkage(s) between the bases. Regarding polypeptides, functionally preserved variants may arise, for example, by conservative
25 and/or non-conservative amino acid substitutions, amino acid analogs, and deletions. The function that is preserved depends upon the relevant function being considered. For example, a YRT2 polynucleotide is considered for its ability to encode a YRT2 polypeptide (or fragment thereof), then the ability of a variant sequence to encode the same polypeptide is the relevant function. If a YRT2 polypeptide is considered for its ability to bind to a
30 particular entity (such as an antibody or ligand), then the ability of a variant sequence to encode a polypeptide with equivalent binding characteristics that is relevant.

"Recombinant," as applied to a polynucleotide or gene, means that the polynucleotide is the product of various combinations of cloning, restriction and/or ligation steps, and other procedures that result in a construct that is distinct from a polynucleotide found in nature.

5 A "vector" is a self-replicating nucleic acid molecule that transfers an inserted nucleic acid molecule into and/or between host cells. The term includes vectors that function primarily for insertion of a nucleic acid molecule into a cell, replication of vectors that function primarily for the replication of nucleic acid, and expression vectors that function for transcription and/or translation of the DNA or RNA. Also included are vectors
10 that provide more than one of the above functions.

"Expression vectors" are defined as polynucleotides which, when introduced into an appropriate host cell, can be transcribed and translated into a polypeptide(s). An expression vector also comprises control elements operatively linked to the encoding region to enable and/or facilitate expression of the polypeptide in the target cell. An
15 "expression system" usually connotes a suitable host cell comprised of an expression vector that can function to yield a desired expression product.

A "host cell" includes an individual cell or cell culture which can be or has been a recipient for vector(s) or for incorporation of nucleic acid molecules and/or proteins. Host cells include progeny of a single host cell, and the progeny may not necessarily be
20 completely identical (in morphology or in genomic or total DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected in vivo with a polynucleotide(s) of this invention.

A "cell line" or "cell culture" denotes eukaryotic cells, derived from higher, multicellular organisms, grown or maintained in vitro. It is understood that the descendants of
25 a cell may not be completely identical (either morphologically, genotypically, or phenotypically) to the parent cell. Cells described as "uncultured" are obtained directly from a living organism, and are generally maintained for a limited amount of time away from the organism (i.e., not long enough or under conditions for the cells to undergo substantial replication).

30 "Heterologous" means derived from (i.e., obtained from) a genotypically distinct entity from the entity to which it is being compared. For example, a polynucleotide may be placed by genetic engineering techniques into a plasmid or vector derived from a different

source, thus becoming a heterologous polynucleotide. A promoter which is linked to a coding sequence with which it is not naturally linked is a heterologous promoter.

An "isolated" or "purified" polynucleotide, polypeptide, antibody or cell is one that is substantially free of the materials with which it is associated in nature. By substantially
5 free is meant at least 50%, preferably at least 70%, more preferably at least 80%, and even more preferably at least 90% free of the materials with which it is associated in nature. As used herein, an "isolated" polynucleotide or polypeptide also refers to recombinant polynucleotides or polypeptides, which, by virtue of origin or manipulation: (1) are not associated with all or a portion of a polynucleotide or polypeptide with which it is
10 associated in nature, (2) are linked to a polynucleotide or polypeptide other than that to which it is linked in nature, or (3) does not occur in nature, or (4) in the case of polypeptides arise from expression of recombinant polynucleotides. Thus, for example, an isolated substance may be prepared by using a purification technique to enrich it from a source mixture. Enrichment can be measured on an absolute basis, such as weight per
15 volume of solution, or it can be measured in relation to a second, potentially interfering substance present in the source mixture. Increasing enrichments of the embodiments of this invention are increasingly more preferred. Thus, for example, a 2-fold enrichment is preferred, 10-fold enrichment is more preferred, 100-fold enrichment is more preferred, 1000-fold enrichment is even more preferred. A substance can also be provided in an
20 isolated state by a process of artificial assembly, such as by chemical synthesis or recombinant expression.

A "stable duplex" of polynucleotides, or a "stable complex" formed between any two or more components in a biochemical reaction, refers to a duplex or complex that is sufficiently long-lasting to persist between formation of the duplex or complex and
25 subsequent detection, including any optional washing steps or other manipulation that may take place in the interim.

A substance is said to be "selective" or "specific" if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. An antibody "specifically
30 binds" to a target if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances.

As used herein, the term "agent" means a biological or chemical compound such as a simple or complex organic or inorganic molecule, a peptide, a protein or an oligonucleotide. A vast array of compounds can be synthesized, for example oligomers, such as oligopeptides and oligonucleotides, and synthetic organic compounds based on various core structures, and these are also included in the term "agent". In addition, various natural sources can provide compounds for screening, such as plant or animal extracts, and the like. Compounds can be tested singly or in combination with one another.

A "polymerase chain reaction" ("PCR") is a reaction in which replicate copies are made of a target polynucleotide using one or more primers, and a catalyst of polymerization, such as a reverse transcriptase or a DNA polymerase, and particularly a thermally stable polymerase enzyme. Methods for PCR are taught in U.S. Patent Nos. 4,683,195 (Mullis) and 4,683,202 (Mullis et al.). All processes of producing replicate copies of the same polynucleotide, such as PCR or gene cloning, are collectively referred to herein as "replication."

An "antibody" (interchangeably used in plural form) is an immunoglobulin molecule capable of specific binding to a target, such as a polypeptide, through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. An antibody can be from any source of animal capable of producing them, for example, mouse, rat, rabbit, or human antibodies. As used herein, the term encompasses not only intact antibodies, but also fragments thereof (such as Fab, Fab', F(ab')₂, Fv, single chain (ScFv)), mutants thereof, fusion proteins, humanized antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity. The term "antibody" includes polyclonal antibodies and monoclonal antibodies.

"Immunological recognition" or "immunological reactivity" refers to the specific binding of a target through at least one antigen recognition site in an immunoglobulin or a related molecule, such as a B cell receptor or a T cell receptor.

The term "antigen" refers to the target molecule that is specifically bound by an antibody through its antigen recognition site. The antigen may, but need not be chemically related to the immunogen that stimulated production of the antibody. The antigen may be polyvalent, or it may be a monovalent hapten. Examples of kinds of antigens that can be

recognized by antibodies include polypeptides, polynucleotides, other antibody molecules, oligosaccharides, complex lipids, drugs, and chemicals.

An "immunogen" is an antigen capable of stimulating production of an antibody when injected into a suitable host, usually a mammal. Compounds may be rendered
5 immunogenic by many techniques known in the art, including crosslinking or conjugating with a carrier to increase valency, mixing with a mitogen to increase the immune response, and combining with an adjuvant to enhance presentation.

General Techniques

10 The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as: "Molecular Cloning: A Laboratory Manual", second edition (Sambrook et al., 1989); "Oligonucleotide Synthesis"
15 (M.J. Gait, ed., 1984); "Animal Cell Culture" (R.I. Freshney, ed., 1987); "Methods in Enzymology" (Academic Press, Inc.); "Handbook of Experimental Immunology" (D.M. Wei & C.C. Blackwell, eds.); "Gene Transfer Vectors for Mammalian Cells" (J.M. Miller & M.P. Calos, eds., 1987); "Current Protocols in Molecular Biology" (F.M. Ausubel et al., eds., 1987); "PCR: The Polymerase Chain Reaction", (Mullis et al., eds., 1994); "Current
20 Protocols in Immunology" (J.E. Coligan et al., eds., 1991); Manipulating the Mouse Embryo, second edition (Hogan et al., 1994); "Modern Biological Analysis of an Organism" (D.C. Shakes, eds., 1995).

Screening methods

25 The present invention encompasses a variety of screening methods using the polynucleotide and/or polypeptide sequences described herein. These methods may be used as a basis for classifying agents in any of a number of ways, including, but not limited to, (a) agents which may exert CNS depressant activity, including candidate anesthetics; (b) agents that may be included in the class of agents in response to which LS and SS mice
30 display differential sensitivity; (c) agents that may be included in the class of agents in response to which there is no differential sensitivity between LS and SS mice. The agents identified and classified under (b) and/or (c) may represent agents which act under two

different kinds of mechanisms. Screening methods of this invention may also be used to identify agents which may modulate CNS depressant activity. Such an agent may be useful, for example, in administering a CNS depressant such as an anesthetic to certain at-risk or overly resistant individuals. Such an agent may also find use in administration to
5 individuals who are overly sensitive to ethanol consumption or who are at risk of developing an addiction to ethanol.

These methods may be practiced in a variety of embodiments. The methods described herein encompass in vitro, cell-based, and in vivo screening assays. In the in vitro embodiments, an agent is tested for its ability to modulate function in a system which
10 does not use intact cells. In the cell-based embodiments, living cells containing sequences described herein are used for testing agents. In the in vivo embodiments, transgenic mice harboring sequences described herein are used for testing agents.

Some of these screening embodiments employ mouse polynucleotide or polypeptide sequences contained in (or encoded in) YAC YRT2, or sequences corresponding to those
15 sequences contained in (or encoded in) YAC YRT2. It is not necessary to know the identity of the sequence(s) (i.e., the individual contiguous nucleotide and/or amino acid identities) to practice these methods. For example, the sequence may be contained in a fragment (such as a restriction fragment or a fragment obtained by PCR) of YRT2, without knowledge of the nucleotide sequence identity of the fragment. Alternatively, a particular
20 known sequence of YRT2 (or known sequence corresponding to a sequence of YRT2) may be used. It is not necessary to obtain sequences for these methods from YRT2 per se, as long as the sequence(s) correspond to those contained in (or encoded in) YRT2.

YRT2 is a YAC containing a 250 kb mouse genomic insert is depicted in Fig. 11 and is described by Schedl et al. (1993) *Nature* 362:258-261. YRT2 was obtained from a
25 YAC library of C3H mouse DNA. Robertson (1987) "Tetracarcinomas and Embryonic Stem Cells" (Robertson, ed.) at 71-112. YRT2 contains an 80kb polynucleotide sequence corresponding to the tyrosinase gene (Fig. 11). We have discovered that the remaining 170 kb contains the gene encoding mGluR5, a metabotropic glutamate receptor (discussed below). There may be other coding and/or control sequences in YRT2. Accordingly,
30 another way of describing the sequences of YRT2, any portion of which may be used in the methods of this invention, are those mouse genomic sequences which encompass the region

corresponding to the entire tyrosinase gene and another approximately 170 kb upstream of the tyrosinase gene.

How generally to prepare any of these sequences or relevant fragments is discussed in a later section addressing polynucleotides and polypeptides of the invention. It is
5 understood that the description of preparation of sequences applies to sequences to be used for the screening methods described herein. Because the genomic location of the mouse sequences of YRT2 are known, and sequences contained within YRT2 are known, one skilled in the art could obtain sequences corresponding to sequences contained within YRT2 from sources other than YRT2 by using, for example, PCR methods applied to a
10 suitable source of mouse DNA (i.e., a source of mouse DNA that would contain the desired sequences), such as commercially available libraries, genomic preparations, cDNA preparations, and DNA obtained by excised gel fragments. Alternatively, because we have shown that the *Lorpl* locus is tightly linked to the tyrosinase gene, these sequences could be used to obtain those and surrounding sequences from a murine genomic (or cDNA) library.
15 Accordingly, mGluR5 sequences (discussed below) and/or tyrosinase sequences may also be used to obtain the desired sequences for these screening methods.

It is also understood that the sequences used in these screening methods can also include regulatory (non-coding) sequences, such as enhancers and/or promoters. When these sequences are used, reporter systems indicating regulatory region function may be
20 employed. Such systems are well-known in the art, and include, but are not limited to, luciferase; aequorin (i.e., green fluorescent protein from *Aequorea victoria*); β -galactosidase; chloramphenicol acetyl transferase; immunologically detectable protein "tags" such as human growth hormone; and the like. See, for example, Current Protocols in Molecular Biology (F.M. Ausubel et al., eds., 1987) and periodic updates. Any assay
25 which detects a product of the reporter gene, either by directly detecting the protein encoded by the reporter gene or by detecting an enzymatic product of a reporter gene-encoded enzyme, is suitable for use in the present invention. Assays include colorimetric, fluorimetric, or luminescent assays or even, in the case of protein tags, radioimmunoassays or other immunological assays. Many of these assays are commercially available.

30 As noted in the definition of "agent" provided above, the agent may be any compound, complex or substance. Generally, the choice of agents to be screened is governed by several parameters, such as the particular polynucleotide or polypeptide target,

its perceived function, its three-dimensional structure (if known or surmised), and other aspects of rational drug design. Techniques of combinatorial chemistry can also be used to generate numerous permutations of candidates. Those of skill in the art can devise and/or obtain suitable agents for testing.

5 An agent is generally identified by its ability to modulate expression of the polynucleotide. As discussed, above, modulation of expression of a polynucleotide may occur at any level that affects its function. An agent may modulate polynucleotide expression by preventing, reducing or increasing transcription. An example of such an agent is one that binds to the upstream controlling region, including a polynucleotide
10 sequence or polypeptide. An agent may modulate expression by preventing, reducing, or increasing translation of its corresponding mRNA. An example of such an agent is one that binds to the mRNA, such as an anti-sense polynucleotide, or an agent which selectively degrades the mRNA, or an agent that selectively stabilizing the mRNA. An agent may modulate expression by binding to the expressed polypeptide. An example of such an
15 agent is a polypeptide or a chelator. Examples of the effect of such binding agents may include the degradation of the polypeptide, increased half-life of the polypeptide, prevention of polypeptide interaction with a ligand, and the stabilization of the polypeptide with a ligand.

 In some embodiments, sequences contained in an mGluR5 gene (including 5' and 3' flanking (non-coding) sequences and introns), which encodes mGluR5, a subclass of
20 metabotropic glutamate receptors, are used. We have discovered evidence that mouse mGluR5 sequences may be involved in this differential response to CNS depressants. For example, a comparison in which LS and SS mice were treated with an agonist of Group I mGluR (which includes mGluR5), ACPD, resulted in a greater change in increased
25 resistance in SS (resistant) mice when compared to LS mice (Example 5). In another experiment, transgenic mice harboring the YAC in an SS background exhibited significantly longer sleep times when treated with an Group I agonist than control (non-transgenic) mice receiving the same agonist (Example 5). These observations suggest that a pathway(s) involving mGluR5 function may play a role in CNS sensitivity (and/or
30 resistance). However, because of the complexity of CNS depressant reaction, and multiple pathways, the inventors are not confining their theory of the sequences associated with the differential CNS depressant response to mGluR5 gene or gene product. Other sequences

on the YAC YRT2 described herein may be associated with this phenomenon, and are included in the screening methods described herein.

Further, because we have observed that mGluR5 appears to play a role in CNS depressant sensitivity in mice, we believe that this receptor plays an analogous functional
5 role on other mammalian species, including human. The high degree of homology among mouse, rat, and human mGluR5 further supports this belief. See SEQ ID NOS:5 and 6, which depict polynucleotide encoding rat mGluR5 (96% overall homology according to a BLAST search comparison) and the amino acid sequence of rat mGluR5 (over 96% overall homology (sequence identity), according to a BLAST search comparison); see also SEQ ID
10 NO:7, which depicts human mGluR5 (over 95% overall homology (sequence identity), according to a BLAST search comparison). Accordingly, the screening methods herein employ mGluR5 sequences, such as mammalian mGluR5, including, but not limited to, mouse, rat, and human. It is also possible, if not likely, that other mGluRs play a part in CNS depressant response. Accordingly, the methods described herein also employ mGluR
15 sequences other than mGluR5, particularly those receptors in class I (mGluR1 and mGluR5 and alternatively spliced variants) and class II (mGluR2 and mGluR3 and alternatively spliced variants). The other mGluR sequences that may be used in these methods include, not only mammalian sequences, but also non-mammalian sequences which show significant (i.e., over 50%) sequence identity to mammalian mGluR sequences. Examples
20 of sources of these other mGluR sequences include, but are not limited to, *C. elegans* (nematode) and *Drosophila*. These sequences are readily available to those skilled in the art, given their publication on public databases such as Genbank and routine methods for obtaining and/or synthesizing polynucleotide sequences.

For any of these mGluR sequences, non-coding as well as coding regions may be
25 used. For example, upstream control regions, such as promoters and/or enhancers, could be obtained and tested. If a non-coding control region is not publicly available on a database such as Genbank, these sequences may be obtained by using routine techniques in the art, such as chromosome walking using publicly available sequences as probes. For example, a cDNA probe containing coding sequences for an mGluR, such as human mGluR5, could be
30 used to probe a human genomic library (or a genomic or cDNA library of another organism, if homologous sequences are desired). Positive, overlapping clones are then analyzed by, for example, restriction analysis. If sequences are desired even further from

the probe sequence, sequentially overlapping clones may be obtained using probes derived from previously identified positive clones, thus effecting walking.

It is also understood that the screening methods of this invention include structural, or rational, drug design, in which the amino acid sequence, three-dimensional atomic
5 structure or other property (or properties) of YRT2 polynucleotide or polypeptide (or mGluR5 polynucleotide or polypeptide) provides a basis for designing an agent which is expected to bind to YRT2 polynucleotide or polypeptide (or mGluR5 polynucleotide or polypeptide). Generally, the design and/or choice of agents in this context is governed by several parameters, such as the perceived function of the YRT2 polynucleotide or
10 polypeptide (or mGluR5 polynucleotide or polypeptide) target, its three-dimensional structure (if known or surmised), and other aspects of rational drug design. Techniques of combinatorial chemistry can also be used to generate numerous permutations of candidate agents.

The screening methods described above represent primary screens, designed, inter
15 alia, to detect any agent that may exhibit CNS depressant activity, or modulate such activity. The skilled artisan will recognize that secondary tests will likely be necessary in order to evaluate an agent further. For example, because a characteristic associated with expression of a YRT2 polynucleotide is generally assessed, it may be desirable to further confirm that this characteristic is involved with CNS depressant activity. One way of
20 accomplishing this would be to conduct the experiment using a known CNS depressant. If the response (i.e., the impact on expression of the YRT2 polynucleotide or mGluR5 polynucleotide) is similar or the same as that response caused by the agent to be tested, it is reasonable to surmise that the characteristic observed is involved in CNS depressant activity.

25 *In vitro embodiments*

In in vitro screening methods of this invention, an agent is screened in an in vitro system, which may include either of the following: (1) an assay for an agent which modulates the translation of a YRT2 (or mGluR5) mRNA or a YRT2 polynucleotide (or an mGluR polynucleotide, including mGluR5) encoding a polypeptide; (2) an assay for an
30 agent that binds to a YRT2 (or mGluR5) polynucleotide(s) or polypeptides (or to a mammalian mGluR polypeptide, including mGluR5).

Accordingly, the invention includes methods of identifying an agent which modulates a YRT2 polynucleotide encoding a YRT2 polypeptide, comprising combining at least one agent to be tested with a YRT2 polynucleotide which encodes a YRT2 polypeptide under suitable reaction conditions, and determining whether the at least one agent modulates this polynucleotide by analyzing at least one characteristic associated with translation of the YRT2 polynucleotide. The invention also includes methods of identifying an agent which binds to a YRT2 polynucleotide or polypeptide, comprising combining at least one agent with a YRT2 polynucleotide or polypeptide under suitable reaction conditions, and determining whether the at least one agent binds to the YRT2 polynucleotide or YRT2 polypeptide. Examples of these types of assays are provided below. These methods also pertain to using mGluR polypeptide(s) and/or polynucleotide(s), such as mammalian mGluR, such as mGluR5, which includes mouse mGluR5. For the discussion which follows, it is understood that the YRT2 sequences serve as example and that these techniques pertain also to mGluR sequences.

For an assay that determines whether an agent modulates the translation of YRT2 mRNA or a polynucleotide encoding a YRT2 polypeptide, an in vitro transcription/translation system may be used. These systems are available commercially and provide an in vitro means to produce mRNA corresponding to a polynucleotide sequence of interest. After YRT2 mRNA is made, it can be translated in vitro and the translation products compared. Comparison of translation products between an in vitro expression system that does not contain any agent (negative control) with an in vitro expression system that does contain an agent indicates whether the agent is affecting translation. Comparison of translation products between control and YRT2 polynucleotides indicates whether the agent, if acting on this level, is selectively affecting translation of the YRT2 mRNA (as opposed to affecting translation in a general, non-selective or non-specific fashion).

In an example for an assay for an agent that binds to a YRT2 polypeptide, a YRT2 polynucleotide encoding a polypeptide is first recombinantly expressed in a prokaryotic or eukaryotic expression system as a native or as a fusion protein in which the YRT2 polypeptide is conjugated with a well-characterized epitope or protein as described under "Preparation of polypeptides of this invention". Recombinant YRT2 polypeptide is then purified by, for instance, immunoprecipitation using anti-YRT2 polypeptide antibodies or

anti-epitope antibodies or by binding to immobilized ligand of the conjugate. An affinity column made of YRT2 polypeptide or YRT2 polypeptide-fusion protein is then used to screen a mixture of compounds which have been appropriately labeled. Suitable labels include, but are not limited to fluorochromes, radioisotopes, enzymes and
5 chemiluminescent compounds. The unbound and bound compounds can be separated by washes using various conditions (e.g. high salt, detergent) that are routinely employed by those skilled in the art. Non-specific binding to the affinity column can be minimized by pre-clearing the compound mixture using an affinity column containing merely the conjugate or the epitope. Similar methods can be used for screening for an agent(s) that
10 competes for binding to YRT2 polypeptides. In addition to affinity chromatography, there are other techniques such as measuring the change of melting temperature or the fluorescence anisotropy of a protein which will change upon binding another molecule. For example, a BIAcore assay using a sensor chip (supplied by Pharmacia Biosensor, Stitt et al. (1995) *Cell* 80: 661-670) that is covalently coupled to native YRT2 polypeptide,
15 fragments thereof, or YRT2 polypeptide-fusion proteins, may be performed to determine the YRT2 polypeptide binding activity of different agents.

It is understood that anti-YRT2 polypeptide antibodies, including anti-mGluR5 antibodies, can be used to determine whether agents which interact with YRT2 polypeptides alter the structure and/or conformation of the YRT2 polypeptide. For
20 example, a conformational change and/or structural alteration induced by contact with an agent may result in the YRT2 polypeptide becoming unrecognizable by the YRT2 polypeptide-specific antibodies. The loss of the ability of a monoclonal anti-YRT2 polypeptide antibody to immunoprecipitate YRT2 polypeptide after the polypeptide has been contacted by the agent would suggest that the agent had interfered, either directly or
25 through a conformational change and/or a structural alteration, with the antibody recognition site on the YRT2 polypeptide. Other ways to assess this interaction are well known in the art. As such a change may alter YRT2 polypeptide function, agents screened for their effect on YRT2 polypeptide - anti-YRT2 polypeptide interactions would be useful for the refinement of those agents known to interact with YRT2 polypeptide to those that
30 may alter YRT2 polypeptide polynucleotide(s) function. Methods for making antibodies are known in the art and need not be described in detail herein.

Cell-based embodiments

These embodiments employ cell systems containing YRT2 and/or mGluR polynucleotides and/or polypeptide(s) (which, as the definitions make clear, include sequences corresponding to YRT2 polynucleotides and/or polypeptides). In one embodiment, the method provides methods for identifying an agent which may exhibit
5 CNS depressant activity, comprising the steps of (a) introducing a polynucleotide comprising a mouse associated with CNS depressant sensitivity into a suitable host cell, wherein the polynucleotide comprises a mouse polynucleotide sequence corresponding to a polynucleotide sequence contained in yeast artificial chromosome YRT2; (b) contacting host cell of step (a) with at least one agent to be tested; (c) analyze at least one
10 characteristic associated with expression of the polynucleotide, wherein an agent is identified by its ability to modulate expression of the mouse YRT2 polynucleotide.

In another embodiment, the invention provides methods for classifying a CNS depressant comprising the steps of (a) introducing a polynucleotide associated with CNS depressant sensitivity into a suitable host cell, wherein the polynucleotide comprises a
15 mouse polynucleotide sequence corresponding to a polynucleotide sequence contained in yeast artificial chromosome YRT2; (b) contacting the host cell with the CNS depressant to be classified; (c) analyzing at least one characteristic associated with expression of the polynucleotide, wherein a CNS depressant which modulates expression may fall within that class of CNS depressants which displays differential reactivity with respect to LS and SS
20 mice.

These methods could also be used to identify an agent which may modulate CNS depressant activity. In another embodiment, the invention provides methods for identifying an agent which may modulate CNS depressant sensitivity in an individual, comprising (a) introducing a polynucleotide associated with CNS depressant sensitivity into a suitable host
25 cell, wherein the polynucleotide comprises a mouse polynucleotide sequence corresponding to a polynucleotide sequence contained in yeast artificial chromosome YRT2, (b) contacting host cell of step (a) with at least one agent to be tested; (c) analyze at least one characteristic associated with expression of the polynucleotide, wherein an agent is identified by its ability to modulate expression of the mouse YRT2 polynucleotide.
30 Preferably, these methods would compare the effect of an agent alone versus the agent in the presence of a CNS depressant (i.e., contacting the cell with the CNS depressant in addition to the agent).

All of these methods preferably include a control sample which does not receive the agent(s). The characteristic(s) to be analyzed may be assessed in any number of ways, including, but not limited to, microscopic analysis, viability testing, ability to replicate, histological examination, the level of a particular RNA or polypeptide associated with the cells, the level of enzymatic activity expressed by the cells or cell lysates, and the ability of the cells to interact with other cells or compounds. Differences between treated and untreated cells indicate effects attributable to the agent(s). Optimally, the agent has a greater effect on experimental cells than on control cells.

To identify agents that specifically modulate YRT2 polynucleotide transcription, for example, the transcription regulatory regions of a gene contained in YRT2 could be linked to a reporter gene and the construct added to an appropriate host cell. As used herein, the term "reporter gene" means a gene that encodes a gene product that can be identified (i.e., a reporter protein). Reporter genes include, but are not limited to, alkaline phosphatase, chloramphenicol acetyltransferase, β -galactosidase, luciferase and green fluorescence protein (GFP). Identification methods for the products of reporter genes include, but are not limited to, enzymatic assays and fluorimetric assays. Reporter genes and assays to detect their products are well known in the art and are described, for example in Ausubel et al. (1987) and periodic updates. Reporter genes, reporter gene assays and reagent kits are also readily available from commercial sources.

Cells transcribing YRT2 mRNA or polynucleotide could be used to identify agents that specifically modulate the half-life of YRT2 mRNA (or polynucleotide) and/or the translation of YRT2 mRNA. Such cells would also be used to assess the effect of an agent on the processing and/or post-translational modification of a YRT2 polypeptide. An agent could modulate the amount of YRT2 polypeptide in a cell by modifying the turn-over (i.e., increase or decrease the half-life) of the YRT2 polypeptide(s). The specificity of the agent with regard to the YRT2 mRNA and polypeptide would be determined by examining the YRT2 products in the absence of the agent and by examining the products of unrelated mRNAs and polypeptides. Methods to examine mRNA half-life, protein processing, and protein turn-over are well known to those skilled in the art.

Cell-based screening methods could also be useful in the identification of agents that modulate YRT2 polypeptide function through the interaction with a YRT2 polypeptide directly. Such agents could block normal YRT2 polypeptide-ligand interactions, if any, or

could enhance or stabilize such interactions. The effect of the agent could be determined using immunoprecipitation reactions. Anti-YRT2 polypeptide antibodies would be used to precipitate YRT2 polypeptide and any protein tightly associated with it. By comparing the polypeptides immunoprecipitated from treated cells and from untreated cells, an agent
5 could be identified that would augment or inhibit YRT2 polypeptide-ligand interactions, if any. YRT2 polypeptide-ligand interactions could also be assessed using cross-linking reagents that convert a close, but noncovalent interaction between polypeptides into a covalent interaction. Techniques to examine protein-protein interactions are well known to those skilled in the art.

10 As discussed above, it is understood that these screening methods also encompass using mGluR sequences, including mammalian mGluR5 from mouse, rat, or human, such as human mGluR5.

For methods using mGluR5 sequences, modulation of mGluR5 activity is measured. Accordingly, an agent may modulate mGluR5 function by affecting any of the following
15 non-limiting examples: transcription; translation; post-translational modification; conformation, including conformation of intact receptor; placement in membrane; cellular localization; interaction with other mGluRs; interaction with ligand; interaction with other moieties; altering a function of a member of a signal transduction pathway.

Any of these levels of mGluR5 function may be measured using methods known in
20 the art. Assays for measuring alterations in transcription, translation, and binding have been discussed above. As further example, modulation of mGluR5 activity may be ascertained by measuring the effects on intracellular calcium, inositol phosphate, and/or cyclic AMP (cAMP).

Intracellular calcium concentration can be measured using methods known in the
25 art, such as fura-2. Briefly, recombinant cells expressing mGluR5 (or a functional fragment thereof) are loaded with fura-2 and suspended in buffer containing 0.5 mM CaCl_2 . An agent(s) is added, and changes in fluorescence signal are measured. Agent(s) may be added over a range of concentrations.

30 Ion-exchange columns eluted with chloride provide a relatively rapid means of screening for inositol phosphate formation. Inositol phosphate may further be studied using HPLC.

cAMP levels may be measured by heating a sample treated with agent in water at about 70°C for about 5-10 minutes. Cellular debris is removed by centrifugation after cycles of freezing and thawing. cAMP concentration is determined by RIA.

Suitable host cells for these methods include any host cell which is capable of
5 accepting and expressing a YRT2 (or mGluR) polynucleotide. Examples of suitable host cells include eukaryotic cells, such as yeast cells (such as, for example *P. pastoris*, *Saccharomyces cerevisiae*, *Candida tropicalis*, *Hansenula polymorpha*, *Schizosaccharomyces pombe* and the like), insect cells, fungal cells, amphibian cells (such as *Xenopus*), nematode cells (such as *C. elegans*) and mammalian cells. Mammalian cells
10 are widely available and need not be discussed in detail herein. Exemplary cells for suitable for practicing these methods include COS cells, mouse L cells, Chinese hamster ovary (CHO) cells, human embryonic kidney (HEK) cells, African green monkey cells, all of mammalian origin; *Xenopus laevis* oocytes, of amphibian origin; *Saccharomyces cerevisiae*, *Pichia pastoris* which are yeast. Exemplary cells for expressing injected RNA
15 transcripts include *Xenopus laevis* oocytes. Methods for injecting *Xenopus* oocytes are well-known in the art. Other, more particular, cell types, well-known to those skilled in the art, include HEK293; Ltk⁺ cells; CDS-7 cells; CHO cells; DG44 cells (dhfr CHO cells; e.g., Urlaub et al. (1986) *Cell. Molec. Genet.* 12:555); BHK cells, and cells of neuronal origin, such as HCN1A and HCN2 (derived from human CNS). Virtually all of these cell types, as
20 well as other suitable cell types, such as neural cells, are either commercially available or are available through the ATCC.

For methods involving mGluR5 sequences, mammalian expression systems, including commercially available systems and other such systems known to those of skill in the art which express G-proteins (either endogenously or recombinantly), for expression of
25 DNA encoding the human metabotropic glutamate receptor subtypes provided herein, are preferred. *Xenopus* oocytes are preferred for expression of in vitro mRNA transcripts of DNA encoding those human metabotropic receptor subtypes that are coupled to the PI hydrolysis/Ca⁺⁺ signalling pathways. An endogenous inositol triphosphate second messenger-mediated pathways in oocytes permits functional expression of human
30 metabotropic receptors in these cells. Oocytes expressing recombinant human metabotropic receptors respond to agonist via the oocyte G-protein-coupled IP₃ generation pathway, which stimulates release of Ca⁺⁺ from internal stores, and reportedly activates a

chloride channel that can be detected as a delayed oscillatory current by voltage-clamp recording. Methods for injecting *Xenopus* oocytes are well-known in the art.

Host cells for functional recombinant expression of human metabotropic receptors preferably express endogenous or recombinant guanine nucleotide-binding proteins (i.e., G-proteins). G-proteins are a highly conserved family of membrane-associated proteins composed of α , β and γ subunits. The α subunit, which binds GDP and GTP, differs in different G-proteins. The attached pair of β and γ subunits may or may not be unique; different α claims may be linked to an identical $\alpha\gamma$ pair or to different pairs. Linder and Gilman (1992) *Sci. Am.* 267:56-65. More than 30 different cDNAs encoding G protein α subunits have been cloned. See, for example, Simon et al. (1991) *Science* 252:802. Four different β polypeptide sequences are known. Simon et al. (1991). Three of five identified γ cDNAs have been cloned. Hurley et al. (1984) *Proc. Natl. Acad. Sci. U.S.A.* 81:6948; Gautam et al. (1989) *Science* 244:971; and Gautam et al. (1990) *Proc. Natl. Acad. Sci. U.S.A.* 87:7973. The sequences of a fourth γ cDNA (Kleuss et al. (1993) *Science* 259:832) and a fifth γ cDNA (Fisher and Aronson (1992) *Mol. Cell. Bio.* 12:1585) have been established, and additional γ subtypes may exist (Tamir et al. (1991) *Biochemistry* 30:3929. G-proteins switch between active and inactive states by guanine nucleotide exchange and GTP hydrolysis. Inactive G protein is stimulated by a ligand activated receptor to exchange GDP for GTP. In the active form, the α subunit, bound to GTP, dissociates from the $\beta\gamma$ complex, and the subunits then interact specifically with cellular effector molecules to evoke a cellular response. Because different G-proteins can interact with different effector systems (e.g., phospholipase C, adenylyl cyclase systems) and different receptors, it is useful to investigate different host cells for expression of different recombinant human metabotropic receptor subtypes. Alternatively, host cells can be transfected with G-protein subunit-encoding DNAs for heterologous expression of differing G proteins.

A practitioner of ordinary skill will be well acquainted with techniques for transfecting eukaryotic cells, including the preparation of a suitable vector, such as a viral vector; conveying the vector into the cell, such as by electroporation; and selecting cells that have been transformed, such as by using a reporter or drug sensitivity element. The effect of an agent on transcription from a YRT2 regulatory region in these constructs would be assessed through the activity of the reporter gene product.

DNA may be stably incorporated into cells or may be transiently expressed using methods known in the art. Stably transfected mammalian cells may be prepared by transfecting cells with an expression vector having a selectable marker gene (such as, for example gene for thymidine kinase, dihydrofolate reductase, neomycin resistance, and the like), and growing the transfected cells under conditions selective for cells expressing the marker gene. To prepare transient transfectants, mammalian cells are transfected with a reporter gene (such as the *E. coli* β -galactosidase gene) to monitor transfection efficiency. Selectable marker genes are typically not included in the transient transfections because the transfectants are typically not grown under selective conditions, and are usually analyzed within a few days after transfection.

In vivo embodiments

The invention also includes methods for identifying agents based on transgenic, or in vivo, systems. For these methods, an agent is administered to a transgenic animal, such as a mouse, harboring, for example, mouse sequences corresponding to YRT2 as described above. The agent is assessed by analyzing a characteristic associated with CNS depressant activity, such as, for example, loss of righting reflex (LORR), or sleep times. Various agents may be assessed for their ability or inability to affect any of these characteristics. Various agents may also be assessed for their ability to alter the effect of a CNS depressant, thus indicating that this agent(s) may modulate CNS depressant activity.

Accordingly, the invention includes methods of identifying an agent which may exhibit CNS depressant activity, comprising the steps of (a) administering an agent to a transgenic animal containing an introduced mouse YRT2 sequence; and (b) analyzing at least one characteristic associated with CNS depressant activity, wherein an agent is identified by its ability to modulate the characteristic. The invention also includes methods of classifying agents and methods of identifying an agent which may modulate CNS depressant activity using these steps. For any of these transgenic embodiments, characteristics which are associated with CNS depressant activity include, but are not limited to, sleep (unconsciousness) time, degree of loss of reflex or response to stimuli, and degree of loss of pain response. Alternatively, the transgenic embodiments may employ mGluR sequences as described above.

Preferably, these methods are performed by comparing the effect of the agent(s) on the transgenic animal described above with the effect on a SS (resistant) mouse. Candidate

CNS depressant agents may also be thus classified by making this comparison with respect to whether they belong in the class of CNS depressants which display differential activity in LS versus SS mice.

In one embodiment, the transgenic animal (such as mouse) contains mGluR5
5 sequences. These mGluR5 sequences may be from a mammal, such as mouse, rat, primate or human. Alternatively, the transgenic animal contains other mGluR sequences, such as mGluR1, mGluR2, mGluR3, mGluR4, mGluR6, mGluR7 and mGluR8 (as well as any splice variants or non-mammalian mGluR homologs). Any of these various mGluR
10 sequences may be from mammalian systems, such as mouse, rat, primate or human, or non-mammalian systems, such as homologous sequences from *Drosophila* and *C. elegans*.

Obtaining these sequences has been described above. A transgenic animal may be mammals, such as mice, rats, primate, cows, pigs, dogs and cats. Alternatively, a transgenic animal may be an invertebrate, such as *Drosophila* or *C. elegans* (nematode). Methods of making transgenic animals, particularly mice, are known in the art. Briefly,
15 polynucleotide sequence(s) are isolated using standard methods of the art. Purified polynucleotide (usually DNA) is injected or introduced into appropriate early cell types or lineages, such as oocytes. See, for example, Brinster et al.(1985) *Proc. Natl. Acad. Sci. USA* 82:4438-4442. Other means known in the art of introducing the polynucleotide are transfection and transduction. Alternatively, appropriate cells may be infected with viruses,
20 such as retroviruses or adenoviruses, which are modified to carry the polynucleotide sequences to be introduced.

Pluripotent stem cells derived from the inner cell mass of the embryo and stabilized in culture can be manipulated in culture to incorporate nucleotide sequences. A transgenic animal can be produced from such stem cells through implantation into a blastocyst that is
25 implanted into a foster mother and allowed to come to term. In the case of mice, injected oocytes are transferred into pseudopregnant females. Animals suitable for transgenic experiments can be obtained from standard commercial sources such as Charles River (Wilmington, MA) and Harlan Sprague Dawley (Indianapolis, IN).

Methods for culturing embryonic stem (ES) cells and the subsequent production of
30 transgenic animals by the introduction of DNA into ES cells using methods such as electroporation, calcium phosphate/DNA precipitation and direct injection are well known in the art, as are procedure for embryo manipulations. Microinjection procedures for fish,

amphibian eggs and birds are detailed by Houdebine and Chourrout (1991) *Experientia* 47:897-905. See also U.S. Pat. 4,945,050.

Transfection and isolation of desired clones can be carried out using standard techniques. For example, random gene integration can be carried out by co-transfecting the nucleic acid with a gene encoding antibiotic resistance. Alternatively, the gene encoding antibiotic resistance is physically linked to a polynucleotide used in the screening methods described herein.

DNA molecules introduced into ES cells can also be integrated into the chromosome through homologous recombination. See, for example, Capecchi (1989) *Science* 244:1288-1292. Methods for positive selection of the recombination event (such as neomycin resistance) and dual positive-negative selection (e.g., neomycin resistance and gancyclovir resistance) and the subsequent identification of desired clones by PCR are methods standard in the art. Capecchi (1989); Joyner et al. (1989) *Nature* 338:153-156.

Targeted ES cells are then injected into blastocysts, which are transferred into pseudopregnant females. Resulting chimeric animals are bred and offspring analyzed by Southern blotting to identify individuals carrying the introduced polynucleotide sequences.

Transgenic mice are identified by preparing DNA from, for example, tail biopsies and simultaneous hybridization with appropriate probes that indicate the presence of introduced DNA. In certain circumstances, presence of introduced DNA (polynucleotide) is indicated by a measurable phenotype, such as change in pigmentation. The transgenic animal (herein exemplified by mouse, due to its more common use) may or may not have more than one copy of the YRT2 sequence(s) or mGluR sequence(s). Depending on the nature of the experiment, it may also be desirable to generate different transgenic lines which have different copy numbers. Copy number can be determined by using methods standard in the art, such as Southern blot analysis or PCR.

The procedure for generating transgenic rats is similar to that of mice. See, for example, Hammer et al. (1990) *Cell* 63:1099-1112. Procedures for production of transgenic non-rodent mammals and other animals are also known in the art. See, for example, Houdebine and Chourrout; Pursel et al. (1989) *Science* 244:1281-1288; Simms et al. (1988) *Bio/Technology* 6:179-183.

If desired, the endogenous mGluR gene may be inactivated. This may be accomplished, for example, by using a recombinant gene engineered to contain an

insertional mutation (such as *neo*). The recombinant gene is inserted into the genome of a recipient cells, tissue, or animal, and transcription of the endogenous mGluR is prevented, or decreased.

5 ***Polynucleotide and polypeptide sequences of the invention***

The invention provides new polynucleotide and polypeptide sequences associated with differential CNS depressant response, namely, a class of CNS depressants defined by a differential response/sensitivity in LS versus SS mice. Examples of these CNS depressants are discussed above. In particular, the invention provides mouse mGluR5
10 polynucleotide and polypeptide sequences. These sequences have a variety of uses, including use in screening methods described above, as indicators of CNS depressant function, and use in obtaining corresponding human sequences which are associated with differential response to CNS depressants, which in turn may provide a useful drug target.

The polynucleotide sequence of most of mGluR5 coding region is depicted in SEQ
15 ID NO:1. The polypeptide sequence of most of mGluR5 is depicted in SEQ ID NO:2.

Bacteria containing respective cloned DNA have been deposited with the American Type Culture Collection (ATCC), 12310 Parklawn Drive, Rockville, Maryland, U.S.A. 20852 on _____, 199_, under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent
20 Procedure. They were accorded Accession Numbers as follows:

Polynucleotides of the invention

The invention provides mGluR5 polynucleotides, which will be described below. The invention also provides vectors containing these polynucleotides, host cells containing
25 these polynucleotides, and compositions comprising these polynucleotides. These polynucleotides are isolated and/or produced by chemical and/or recombinant methods, or a combination of these methods. Unless specifically stated otherwise, "polynucleotides" shall include all embodiments of the polynucleotides of this invention. It is also understood that, all polynucleotide embodiments are isolated polynucleotides. The cloning of mGluR5
30 polynucleotide sequences is described in Example 4. Examination of Genbank using a BLAST search revealed that a portion of mouse mGluR5, represented by an exon, had previously been obtained (SEQ ID NO:3). However, the conceptual translation of this

region does not correspond to the conceptual amino acid translation of SEQ ID NO:1, which is depicted in SEQ ID NO:2.

Accordingly, this invention provides an isolated polynucleotide that contains a sequence encoding a mouse mGluR5 polypeptide wherein the polypeptide is at least about
5 10 amino acids in length and is depicted in SEQ ID NO:2 but not depicted in SEQ ID NO:6 (rat mGluR5 amino acid sequence) or SEQ ID NO:7 (human mGluR5 amino acid sequence). In other embodiments, the mouse mGluR5 encoded is at least about 20, at least about 25, at least about 50, at least about 75, at least about 100, at least about 125, at least about 200, at least about 250, at least about 300, at least about 350, at least about 400, at
10 least about 500, at least about 700, at least about 750, at least about 1000 amino acids in length and depicted in SEQ ID NO:2 but not depicted in SEQ ID NO:6 or SEQ ID NO:7. The invention also includes an isolated polynucleotide comprising a polynucleotide encoding the polypeptide of SEQ ID NO:2.

In other embodiments, the invention includes an isolated polynucleotide comprising
15 a polynucleotide of at least about 10 contiguous nucleotides of SEQ ID NO:1, wherein the about 10 contiguous nucleotides are not depicted in SEQ ID NO:3 (the above-discussed mouse mGluR5 sequence on the Genbank database) or SEQ ID NO:5 (rat mGluR5 polynucleotide sequence). Alternatively, the invention includes an isolated polynucleotide comprising a polynucleotide of at least about 10 contiguous nucleotides of nucleotide 1 to
20 2625 SEQ ID NO:1, wherein the 10 contiguous nucleotides are not depicted in SEQ ID NO:5. The invention also includes an isolated polynucleotide comprising a polynucleotide of at least about 10 contiguous nucleotides of nucleotide 2726 to 3591 of SEQ ID NO:1, wherein the 10 contiguous nucleotides are not depicted in SEQ ID NO:5. Alternatively, the isolated polynucleotide comprises a polynucleotide of at least about 25, at least about 50, at
25 least about 75, at least about 100, at least about 150, at least about 200, at least about 250, at least about 300, at least about 500, at least about 750, at least about 1000 contiguous nucleotides of SEQ ID NO:1, wherein the contiguous nucleotides are not depicted in SEQ ID NO:5. In another embodiment, the invention includes a polynucleotide comprising the sequence of SEQ ID NO:1. The shorter embodiments are useful, for example, as probes as
30 well as providing a template for production of mGluR5 polypeptide(s) and/or functional fragments.

The invention includes modifications to the mGluR5 polynucleotides described above such as deletions, substitutions, additions, or changes in the nature of any nucleic acid moieties. A "modification" is any difference in nucleotide sequence as compared to a polynucleotide shown herein to encode an mGluR5 polypeptide, and/or any difference in terms of the nucleic acid moieties of the polynucleotide(s). Such changes can be useful to facilitate cloning and modifying expression of mGluR5 polynucleotides. Such changes also can be useful for conferring desirable properties to the polynucleotide(s), such as stability. The definition of polynucleotide provided herein gives examples of these modifications. Hence, the invention also includes functionally-preserved variants of the nucleic acid sequences disclosed herein, which include nucleic acid substitutions, additions, and/or deletions.

The invention also encompasses mGluR5 polynucleotides including full-length (unprocessed), processed, coding, non-coding (including flanking region) or portions thereof, provided that these polynucleotides contain a region encoding at least a portion of mGluR5. Also embodied are the mRNA and cDNA sequences and fragments thereof that include a portion mGluR5 encoding segment.

The invention also encompasses polynucleotides encoding for functionally equivalent variants and derivatives of full-length mGluR5 and functionally equivalent fragments thereof which may enhance, decrease or not significantly affect properties of the polypeptides encoded thereby. For instance, changes in a DNA sequence that do not change the encoded amino acid sequence, as well as those that result in conservative substitutions of amino acid residues, non-deleterious non-conservative substitutions, one or a few amino acid deletions or additions, and substitution of amino acid residues by amino acid analogs are those which will not significantly affect properties of the encoded polypeptide. Nucleotide substitutions that do not alter the amino acid residues encoded can be useful for optimizing gene expression in different systems. Suitable substitutions are known to those of skill in the art and are made, for instance, to reflect preferred codon usage in the particular expression systems. In another example, alternatively spliced polynucleotides can give rise to a functionally equivalent fragment or variant of mGluR5. Alternatively processed polynucleotide sequence variants are defined as polynucleotide sequences corresponding to mRNAs that differ in sequence for one another but are derived from the same genomic region, for example, mRNAs that result from: 1) the use of

alternative promoters; 2) the use of alternative polyadenylation sites; and/or 3) the use of alternative splice sites.

The mGluR5 polynucleotides of the invention also include polynucleotides encoding other mGluR5 fragments. The polynucleotides encoding mGluR5 fragments are useful, for example, as probes, therapeutic agents, a polypeptide processing signal, and as a template for encoding various functional domains of mGluR5. Accordingly, the invention includes a polynucleotide that comprises a region of at least 15 contiguous nucleotides, more preferably at least about 20 contiguous nucleotides, more preferably at least about 25 contiguous nucleotides, more preferably at least about 35 contiguous nucleotides, more preferably at least about 50 contiguous nucleotides, even more preferably at least about 75 contiguous nucleotides, even more preferably at least about 100 contiguous nucleotides, even more preferably at least about 200 contiguous nucleotides, even more preferably at least about 300 contiguous nucleotides, even more preferably at least about 500 contiguous nucleotides, even more preferably at least about 750 contiguous nucleotides, even more preferably at least about 1000 contiguous nucleotides, even more preferably at least about 1250 contiguous nucleotides, of SEQ ID NO:1, provided that the contiguous nucleotides are not depicted in SEQ ID NO:3 or SEQ ID NO:5.

Another embodiment of the invention is isolated polynucleotides comprising a region of at least about 20 contiguous nucleotides, with the region having at least 97% sequence identity with a sequence depicted in SEQ ID NO:1. Alternatively, the region may also have 98% sequence identity, preferably 98.5% sequence identity, preferably 99% sequence identity. Alternatively, these regions may comprise at least about 25 contiguous nucleotides, at least about 30 contiguous nucleotides, at least about 50 contiguous nucleotides, at least about 60 contiguous nucleotides, at least about 75 contiguous nucleotides, at least about 100 contiguous nucleotides, at least about 200 contiguous nucleotides, at least about 250 contiguous nucleotides, at least about 300 contiguous nucleotides, at least about 500 contiguous nucleotides, at least about 700 contiguous nucleotides, at least about 1000 contiguous nucleotides, any and each of which can have any of the above-specified degrees of sequence identity. It is understood that these contiguous nucleotide sequences are not depicted in SEQ ID NO:3 or SEQ ID NO:5.

As known to those of skill in the art, methods for determination of sequence identity between two polynucleotides can include the use of hybridization reactions and sequence

alignment algorithms. Alignment of known polynucleotide sequences relative to each other with commercially available sequence comparison programs, such as BLAST programs or those found in the GCG, Inc. software packages, provide a means to directly compare sequences for degree of identity. Such programs establish the sequence alignments and can
5 provide the percentage of sequence identity between them.

Generally, such programs identify the best sequence alignment by scoring the alignment for matches, mismatches, and gaps in the sequence created in the generation of the alignment. For example, the BESTFIT program from GCG uses the algorithm of Smith and Waterman with the default scoring of a match value of 10, a mismatch value of -9, a
10 gap creation penalty of 50 and a gap extension penalty of 3. Thus, the quality score for a polynucleotide alignment can be determined using the equation:

$$\text{Quality} = (10 \times \text{matches}) + (-9 \times \text{mismatches}) - (50 \times \text{gap number}) - (3 \times \text{total length of gaps})$$

Other alignment programs are based on other algorithms which score alignment with
15 different values (e.g., the GAP program from GCG uses the Needleman and Wunsch algorithm with the default match and gap scoring values as described for the BESTFIT program but a default mismatch value of 0 and the FASTA program uses a Pearson and Lipman search with a default gap creation penalty of 16 and gap extension penalty of 4).

Similar alignment programs can be used to assess amino acid sequence similarity
20 between polypeptide sequences. Often, the alignment of polypeptide sequences are scored using values different than those used with polynucleotides. For example, the FASTA program uses a default gap creation penalty of 12 and gap extension penalty of 2. Once the polypeptides are aligned relative to each other, the programs can provide the degree of amino acid similarity and identity.

25 Hybridization can also be used in the determination of polynucleotide sequence identity. The stability of hybridized sequences is reflected in the melting temperature (T_m ; discussed below) of the hybrids. For polynucleotides greater than 100 nucleotides in length, the T_m can be used to approximate the degree of sequence identity between two sequences because the T_m appears to decrease 0.5 - 1.5 °C for every 1% base pair mismatch
30 in the hybrid. This approach can only approximate the degree of identity because the base composition of and mismatch distribution in the hybrids influence the actual change in T_m .

Generally, to determine the approximate sequence identity between two polynucleotide sequences with hybridization, the lowest temperature at which hybridization occurs between identical sequences is established for a particular hybridization solution (see below) and the T_m is determined. When the polynucleotide sequence is then
5 hybridized with another sequence, a decrease in the T_m of the reaction can be correlated to the degree of mismatch between the sequences as described above (i.e., roughly 1% degree of mismatch reduces the T_m of a heteroduplex by 0.5 - 1.5 °C).

Hybridization reactions can be performed under conditions of different "stringency". Stringency of hybridization is used herein to refer to conditions under which
10 polynucleotide hybrids are stable. Conditions that increase stringency of a hybridization reaction of widely known and published in the art. See, for example, Sambrook et al. (1989) and Ausubel et al. (1987).

" T_m " is the temperature in degrees Centigrade at which 50% of a polynucleotide duplex made of complementary strands hydrogen bonded in anti-parallel direction by
15 Watson-Crick base pairing dissociates into single strands under conditions of the experiment. T_m may be predicted according to a standard formula, such as:

$$T_m = 81.5 + 16.6 \log[X^+] + 0.41 (\%G/C) - 0.61 (\%F) - 600/L$$

where $[X^+]$ is the cation concentration (usually sodium ion, Na^+) in mol/L; (%G/C) is the number of G and C residues as a percentage of total residues in the duplex; (%F) is
20 the percent formamide in solution (wt/vol); and L is the number of nucleotides in each strand of the duplex. Thus, hybrid stability is dependent upon a number of factors including, but not limited to, ionic strength of the hybridization and wash solutions, base composition of the polynucleotides involved in the duplex; destabilizing agents in the hybridization solution (e.g., formamide or urea), and length of the duplex formed.

25 In terms of hybridization conditions, the higher the sequence identity required, the more stringent are the hybridization conditions if such sequences are determined by their ability to hybridize to a sequence of SEQ ID NO:1. Accordingly, the invention also includes polynucleotides that are able to hybridize to a sequence comprising at least 20 contiguous nucleotides (or more, such as 25, 35, 50, 75 or 100 contiguous nucleotides) of
30 SEQ ID NO:1. The hybridization conditions would be stringent, e.g., 80°C (or higher temperature) and 6 X SSC (or less concentrated SSC).

Examples of relevant conditions include (in order of increasing stringency): incubation temperatures of 25°C, 37°C, 50°C and 68°C; buffer concentrations of 10 X SSC, 6 X SSC, 1 X SSC, 0.1 X SSC (where 1 X SSC is 0.15 M NaCl and 15 mM citrate buffer) and their equivalents using other buffer systems; formamide concentrations of 0%,
5 25%, 50%, and 75%; incubation times from 24 hours to 5 minutes; 1, 2, or more washing steps; wash incubation times of 1, 2, or 15 minutes; and wash solutions of 6 X SSC, 1 X SSC, 0.1 X SSC, or deionized water, in temperatures of 25°C, 37°C, 50°C and 68°C.

Accordingly, the invention includes an isolated polynucleotide capable of hybridizing under moderately stringent conditions, more preferably under stringent
10 conditions, to a region of SEQ ID NO:1, wherein the region is at least about 10 contiguous nucleotides. The region may also be at least about 25 contiguous nucleotides, at least about 50 contiguous nucleotides, at least about 100 contiguous nucleotides, at least about 150 contiguous nucleotides, at least about 200 contiguous nucleotides, at least about 200 contiguous nucleotides, at least about 300 contiguous nucleotides, at least about 500
15 contiguous nucleotides, at least about 1000 contiguous nucleotides of a region of SEQ ID NO:1. The invention also includes an isolated polynucleotide capable of hybridizing under moderately stringent conditions, even more preferably under stringent conditions, to the sequence depicted in SEQ ID NO:1.

Compositions containing mGluR5 polynucleotides are encompassed by this
20 invention. The invention also provides compositions comprising a vector(s) containing an mGluR5 polynucleotide as well as compositions comprising a host cell containing an mGluR5 polynucleotide, as described herein. Generally, the compositions further contain a physiologically acceptable medium, such as water, buffered solutions, or pharmaceutically acceptable excipient. These media are well-known in the art.

25 Preparation of mGluR5 polynucleotides of this invention

The polynucleotides of this invention can be obtained using chemical synthesis, recombinant methods, or PCR.

Methods of chemical polynucleotide synthesis are well known in the art and need not be described in detail herein. One of skill in the art can use the sequences provided
30 herein and a commercial DNA synthesizer to produce a desired DNA sequence.

For preparing mGluR5 polynucleotides using recombinant methods, a polynucleotide comprising a desired sequence can be inserted into a suitable vector, and the

vector in turn can be introduced into a suitable host cell for replication and amplification. Polynucleotides may be inserted into host cells by any means known in the art. Cells are transformed by introducing an exogenous polynucleotide by direct uptake, endocytosis, transfection, F-mating or electroporation. Once introduced, the exogenous polynucleotide
5 can be maintained within the cell as a non-integrated vector (such as a plasmid) or integrated into the host cell genome. The polynucleotide so amplified can be isolated from the host cell by methods well known within the art. See, e.g., Sambrook et al. (1989).

Alternatively, PCR allows reproduction of DNA sequences. PCR technology is well known in the art and is described in U.S. Pat. Nos. 4,683,195, 4,800,159, 4,754,065
10 and 4,683,202, as well as *PCR: The Polymerase Chain Reaction*, Mullis et al. eds., Birkauser Press, Boston (1994).

RNA can be obtained by using the isolated DNA in an appropriate vector and inserting it into a suitable host cell. When the cell replicates and the DNA is transcribed into RNA, the RNA can then be isolated using methods well known to those of skill in the art, as set forth in Sambrook et al., (1989), for example. RNA can also be obtained through
15 in vitro reactions. For example, the mGluR5 polynucleotide can be inserted into a vector that contains appropriate transcription promoter sequences. Commercially available RNA polymerases will specifically initiate transcription at their promoter sites and continue the transcription process through the adjoining DNA polynucleotides. Placing the mGluR5
20 polynucleotides between two such promoters allows the generation of sense or antisense strands of mGluR5 RNA.

If used as a vaccine (i.e., pharmaceutical composition for eliciting an immune response), plasmids containing mGluR5 polynucleotides are preferably prepared as described by Horn et al. ((1995) *Human Gene Therapy* 6:565-573) which produces a
25 pharmaceutical grade plasmid DNA suitable for administration.

Cloning and expression vectors comprising mGluR5 polynucleotide

The present invention further includes a variety of vectors containing mGluR5 polynucleotides of this invention. These vectors can be used for expression of recombinant polypeptides as well as a source of mGluR5 polynucleotides. Cloning vectors can be used
30 to obtain replicate copies of the mGluR5 polynucleotides they contain, or as a means of storing the polynucleotides in a depository for future recovery. Expression vectors (and host cells containing these expression vectors) can be used to obtain polypeptides produced

from the polynucleotides they contain. They may also be used where it is desirable to express mGluR5 polypeptides in an individual, such as for eliciting an immune response via the polypeptide(s) encoded in the expression vector(s). Suitable cloning and expression vectors include any known in the art, e.g., those for use in in vitro, bacterial, mammalian, yeast and insect expression systems. Specific vectors and suitable host cells are known in the art and need not be described in detail herein. For example, see Gacesa and Ramji, *Vectors*, John Wiley & Sons (1994).

Cloning and expression vectors typically contain a selectable marker (for example, a gene encoding a protein necessary for the survival or growth of a host cell transformed with the vector), although such a marker gene can be carried on another polynucleotide sequence co-introduced into the host cell. Only those host cells into which a selectable gene has been introduced will survive and/or grow under selective conditions. Typical selection genes encode protein(s) that (a) confer resistance to antibiotics or other toxins substances, e.g., ampicillin, neomycin, methotrexate, etc.; (b) complement auxotrophic deficiencies; or (c) supply critical nutrients not available from complex media. The choice of the proper marker gene will depend on the host cell, and appropriate genes for different hosts are known in the art. Cloning and expression vectors also typically contain a replication system recognized by the host.

Suitable cloning vectors may be constructed according to standard techniques, or may be selected from a large number of cloning vectors available in the art. While the cloning vector selected may vary according to the host cell intended to be used, useful cloning vectors will generally have the ability to self-replicate, may possess a single target for a particular restriction endonuclease, and/or may carry genes for a marker that can be used in selecting clones containing the vector. Suitable examples include plasmids and bacterial viruses, e.g., pUC18, mp18, mp19, pBR322, pMB9, ColE1, pCR1, RP4, phage DNAs, and shuttle vectors such as pSA3 and pAT28. These and many other cloning vectors are available from commercial vendors such as BioRad, Stratagene, and Invitrogen.

Expression vectors generally are replicable polynucleotide constructs that contain a polynucleotide encoding an mGluR5 polypeptide of interest. The mGluR5 polynucleotide encoding the polypeptide is operatively linked to suitable transcriptional controlling elements, such as promoters, enhancers and terminators. For expression (i.e., translation), one or more translational controlling elements are also usually required, such as ribosome

binding sites, translation initiation sites, and stop codons. These controlling elements (transcriptional and translational) may be derived from the mGluR5 gene, or they may be heterologous (i.e., derived from other genes and/or other organisms). A polynucleotide sequence encoding a signal peptide can also be included to allow an mGluR5 polypeptide to cross and/or lodge in cell membranes or be secreted from the cell. A number of expression vectors suitable for expression in eukaryotic cells including yeast, avian, and mammalian cells are known in the art. Another example of an expression vector (system) is the baculovirus/insect cell system. Expression of mGluR5 RNA in vitro is described above.

The vectors containing the polynucleotides of interest can be introduced into the host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (where the vector is an infectious agent, such as vaccinia virus). The choice of means of introducing vectors or mGluR5 polynucleotides will often depend on the host cell.

Host cells transformed with mGluR5 polynucleotides

Another embodiment of this invention are host cells transformed with (i.e., comprising) mGluR5 polynucleotides and/or vectors having mGluR5 polynucleotide(s) sequences, as described above. Both prokaryotic and eukaryotic host cells may be used. Prokaryotic hosts include bacterial cells, for example *E. coli*, *B. subtilis*, and mycobacteria. Among eukaryotic hosts are yeast, insect, avian, plant, *C. elegans* (or nematode) and mammalian cells. Host systems are known in the art and need not be described in detail herein. Examples of fungi (including yeast) host cells are *S. cerevisiae*, *Kluyveromyces lactis* (*K. lactis*), species of *Candida* including *C. albicans* and *C. glabrata*, *Aspergillus nidulans*, *Schizosaccharomyces pombe* (*S. pombe*), *Pichia pastoris*, and *Yarrowia lipolytica*. Examples of mammalian cells are COS cells, house L cells, Chinese hamster ovary (CHO) cells, human embryonic kidney (HEK) cells, African green monkey cells. *Xenopus laevis* oocytes, or other cells of amphibian origin, may also be used.

The host cells of this invention can be used, *inter alia*, as repositories of mGluR5 polynucleotides and/or vehicles for production of mGluR5 polynucleotides and/or polypeptides. They may also be used in the screening methods described above.

Polypeptides of the invention

The present invention encompasses mouse mGluR5 polypeptide sequences. Unless specifically stated, the term "polypeptide(s)" shall include all polypeptide embodiments of this invention. It is also further understood that polypeptide embodiments of this invention are
5 isolated polypeptides.

The polypeptides have a variety of uses, including their use in making antibodies that bind to these polypeptides, their use as agents to screen pharmaceutical candidates (both in vitro and in vivo), and their use in rational (i.e., structure-based) drug design. The mGluR5 polypeptides may also be used to identify proteins that interact physically with mGluR5
10 which could thus themselves be drug targets.

The amino acid sequence of mGluR5 is shown in SEQ ID NO:2. The publicly available conceptual translation of SEQ ID NO:3 (depicted in SEQ ID NO:4) does not correspond to any region of the conceptual translation of SEQ ID NO:1 (as depicted in SEQ ID NO:2).

15 In one embodiment, the invention includes an isolated polypeptide comprising at least about 5 contiguous amino acids of the sequence of SEQ ID NO:2, wherein the about 5 contiguous amino acids are not depicted in SEQ ID NO:6 or in SEQ ID NO:7, and wherein the isolated polypeptide exhibits an mGluR5 function. In this context, "mGluR5 function" includes, not only those functions delineated in the "Definitions" section of this application,
20 but other functions such as ability to elicit an immune response, including a humoral and/or cellular immune response. In other embodiments, the isolated polypeptide comprises at least about 10 contiguous amino acids, at least about 15 contiguous amino acids, at least about 20 contiguous amino acids, at least about 25 contiguous amino acids, at least about 30 contiguous amino acids, at least about 50 contiguous amino acids, at least about 75
25 contiguous amino acids, at least about 100 contiguous amino acids, at least about 150 contiguous amino acids, at least about 200 contiguous amino acids, at least about 250 contiguous amino acids, at least about 300 contiguous amino acids, at least about 400 contiguous amino acids, at least about 500 contiguous amino acids of the sequence of SEQ ID NO:2. In any or all of these instances, the contiguous amino acids are not depicted in
30 SEQ ID NO:6 or SEQ ID NO:7.

In another embodiment, the invention includes an isolated polypeptide comprising an mGluR5 polypeptide, wherein the polypeptide comprises the sequence of SEQ ID NO:2.

5 In another embodiment, the invention provides an isolated polypeptide comprising at least 10 contiguous amino acids which have at least 97% sequence identity to a sequence depicted in SEQ ID NO:2. In other embodiments, the isolated polypeptide comprises at least at least about 15 contiguous amino acids, at least about 20 contiguous amino acids, at least about 25 contiguous amino acids, at least about 30 contiguous amino acids, at least about 50 contiguous amino acids, at least about 75 contiguous amino acids, 10 at least about 100 contiguous amino acids, at least about 150 contiguous amino acids at least about 200 contiguous amino acids, at least about 250 contiguous amino acids, at least about 300 contiguous amino acids, at least about 400 contiguous amino acids, at least about 500 contiguous amino acids, any of which having at least a 98%, preferably at least about 98.5%, preferably at least about 99% sequence identity to a sequence of SEQ ID NO:2. In 15 any or all of these instances, the contiguous amino acids are not depicted in SEQ ID NO:6 or SEQ ID NO:7.

Determination of sequence identity in the context of polypeptide sequences generally involves alignment programs readily and commercially available, and have been discussed above.

20 The size of mGluR5 polypeptides may vary widely, as the length required to effect activity could be as small as, for example, a 5- or 10-mer amino acid sequence to effect binding and trigger a response. The maximum length generally is not detrimental to effecting activity. The minimum size must be sufficient to provide a desired function. Thus, the invention includes polypeptide fragments of mGluR5 comprising a portion of the amino acid 25 sequence depicted in SEQ ID NO:2 in which the mGluR5 polypeptide is about 15, preferably 25, more preferably 50 more preferably 75, more preferably 100 amino acids in length. These lengths could depend on the proposed use of the mGluR5 polypeptide; for example, a polypeptide comprising the transmembrane domain of mGluR5 could be desired, or the extracellular and/or intracellular domain. Based on the amino acid sequence, and what is 30 known about domains of other mGluRs, these domains could be estimated. Alternatively, it may be that only a partial extracellular domain is necessary if binding of glutamate is the

desired function. As noted above, only a relatively small polypeptide could be used if binding to antibody or eliciting an immune response is desired.

As is evident to one skilled in the art, these mGluR5 polypeptides, regardless of their size, may also be associated with, or conjugated with, other substances or agents to facilitate, enhance, or modulate function and/or specificity of an mGluR5 polypeptide.

The invention includes modifications to mGluR5 polypeptides including functionally equivalent fragments of the mGluR5 polypeptides which do not significantly affect their properties and variants which have enhanced or decreased activity. Collectively, these modifications may be termed "analogs" of mGluR5 or a fragment of mGluR5. Modification of polypeptides is routine practice in the art and need not be described in detail herein. Examples of modified polypeptides include polypeptides with conservative substitutions of amino acid residues, one or more deletions or additions of amino acids which do not significantly deleteriously change the functional activity, or use of chemical analogs. Amino acid residues which can be conservatively substituted for one another include but are not limited to: glycine/alanine; valine/isoleucine/leucine; asparagine/glutamine; aspartic acid/glutamic acid; serine/threonine; lysine/arginine; and phenylalanine/tyrosine. Such conservative substitutions are known in the art, and preferably, the amino acid substitutions would be such that the substituted amino acid would possess similar chemical properties as that of the original amino acid. These polypeptides also include glycosylated and non-glycosylated polypeptides, as well as polypeptides with other post-translational modifications, such as, for example, glycosylation with different sugars, acetylation, and phosphorylation. Amino acid modifications can range from changing or modifying one or more amino acids to complete redesign of a region. Other methods of modification include using coupling techniques known in the art, including, but not limited to, enzymatic means, oxidative substitution and chelation. Modifications can be used, for example, for attachment of labels for immunoassay, such as the attachment of radioactive moieties for radioimmunoassay. Modified mGluR5 polypeptides are made using established procedures in the art and can be screened using standard assays known in the art.

The invention also encompasses fusion proteins comprising one or more mGluR5 polypeptides. For purposes of this invention, an mGluR5 fusion protein contains one or more mGluR5 polypeptides and another amino acid sequence to which it is not attached in

the native molecule, for example, a heterologous sequence or a homologous sequence from another region. Useful heterologous sequences include, but are not limited to, sequences that provide for secretion from a host cell, enhance immunological reactivity, or facilitate the coupling of the polypeptide to an immunoassay support or a vaccine carrier. For instance, an mGluR5 polypeptide can be fused with a bioresponse modifier. Examples of bioresponse modifiers include, but are not limited to, cytokines or lymphokines such as GM-CSF, interleukin-2 (IL-2), interleukin 4 (IL-4), and γ -interferon. Accordingly, the invention includes mGluR5 fusion polypeptides that contain GM-CSF or IL-2. Another useful heterologous sequence is one which facilitates purification. Examples of such sequences are known in the art and include those encoding epitopes such as Myc, HA (derived from influenza virus hemagglutinin), His-6, or FLAG. Other heterologous sequences that facilitate purification are derived from proteins such as glutathione S-transferase (GST), maltose-binding protein (MBP), or the Fc portion of immunoglobulin. Another useful heterologous sequence is a functional domain which can substitute for a functional domain of mGluR5. For example, a chimeric receptor could be constructed in order to functionally isolate aspects of mGluR5. See, for example, WO 97/05252.

In another embodiment, mGluR5 polypeptides can be conjugated with carrier or label. For example, in instances where the mGluR5 polypeptide is correctly configured so as to provide a binding site, but is too small to be immunogenic, the polypeptide may be linked to a suitable carrier. A number of techniques for obtaining such linkage are known in the art and need not be described in detail herein. Any carrier can be used which does not itself induce the production of antibodies harmful to the host. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins; polysaccharides, such as latex functionalized sepharose, agarose, cellulose, cellulose beads and the like; polymeric amino acids, such as polyglutamic acid, polylysine, and the like; amino acid copolymers; and inactive virus particles or attenuated bacteria, such as Salmonella. Especially useful protein substrates are serum albumins, keyhole limpet hemacyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, tetanus toxoid, and other proteins well known to those of skill in the art. Labels are known in the art and are described herein.

Compositions containing mGluR5 polypeptides are also encompassed by this invention. When these compositions are to be used pharmaceutically, they are combined

with a pharmaceutically acceptable excipient. The compositions may alternatively or additionally contain a physiologically acceptable medium, examples of which have been provided above.

5 mGluR5 polypeptides of the invention can be identified and/or characterized in a number of ways. For example, an mGluR5 polypeptide can be tested for its ability to bind to, for instance, another protein (such as an antibody or ligand, such as glutamate). Alternatively, mGluR5 polypeptide(s) can be tested for its ability to elicit an immune response, whether humoral or cellular. It is understood that only one of these properties need be present in order for a polypeptide to come within this invention, although more
10 than one of these properties may be present.

The ability of an mGluR5 polypeptide to bind (i.e., interact with) another protein can be assessed using standard techniques in the art. Binding of an mGluR5 polypeptide to an antibody may be assessed, for example, by RIA (i.e., by reacting radiolabeled mGluR5 polypeptide with an antibody that is coated on microtiter plates). In another procedure,
15 binding to an antibody is determined by competitive immunoassay. For example, a fragment is tested for its ability to interfere with the binding between the antibody and another polypeptide known to bind to the antibody. This assay may be conducted by labeling one of the components (i.e., antibody or polypeptide known to bind to the antibody), and optionally immobilizing the other member of the binding pair on a solid
20 support for ease of separation. The test fragment is incubated with labeled region, and then the mixture is presented to the immobilized target to determine if the test fragment is able to inhibit binding.

In the case of testing whether the mGluR5 polypeptide binds to another protein, for instance, a ligand, assays to detect binding are known in the art and need not be described
25 in detail herein. For instance, a protein is immobilized on a suitable column. Extracts or solutions containing the test mGluR5 polypeptide are then run through the column, and a determination is made whether the mGluR5 polypeptide was retained on the column. Conversely, the mGluR5 polypeptides can be immobilized on a column and cell extracts or lysates are allowed to run through the column. Alternatively, the two hybrid technique can
30 be used to identify polypeptides that interact with mGluR5 polypeptides (as well as, the cDNAs that encode such polypeptides) and to test such interactions. Brent et al. U.S. Pat. No. 5,580,736.

For characterizing an mGluR5 polypeptide for its ability to elicit an immune response (whether humoral or cellular) in an individual, standard assays exist in the art. For instance, the ability of an mGluR5 polypeptide to generate a humoral response can be determined by testing for the presence of an antibody that binds to the mGluR5 polypeptide(s) after administration of the mGluR5 polypeptide(s). It is understood that this antibody was not present, or was present in lower amounts, before administration of the mGluR5 polypeptide(s). Immunogenicity is preferably tested in individuals without a previous anti-mGluR5 response. Examples of suitable individual include, but are not limited to, mice, rats, rabbits, goats, monkeys and humans. For this test, an individual is administered an mGluR5 polypeptide(s). The amount per administration and the number of administrations will vary, depending on the individual. Presence of an antibody elicited in response to administration of an mGluR5 polypeptide(s) is determined by standard assays in the art, such as ELISA or RIA. mGluR5 polypeptide(s) may be further characterized by their ability to elicit an antibody that is cytotoxic, or to elicit an antibody that participates in an ADCC response using standard assays in the art.

A mGluR5 polypeptide can also be characterized by its ability to elicit a cellular immune response, using, for example, assays that detect proliferation of peripheral blood mononuclear cells (PBMs) incubated with an mGluR5 polynucleotide. Another way of detecting a cellular immune response is to test for T cell cytotoxicity (CTL) activity. Both of these responses are detected using standard assays in the art.

Other measurable activities may be used to characterize an mGluR5 polypeptide, such as membrane association, particular conformational change upon binding agonist(s) or antagonist(s), and ability to prevent or reduce signal transduction when expressed in a suitable host cell.

Preparation of polypeptides of this invention

The polypeptides of this invention can be made by procedures known in the art. The polypeptides can be produced by recombinant methods (i.e., single or fusion polypeptides) or by chemical synthesis. Polypeptides, especially shorter polypeptides up to about 50 amino acids, are conveniently made by chemical synthesis. Methods of chemical synthesis are known in the art and are commercially available. For example, a polypeptide could be produced by an automated polypeptide synthesizer employing the solid phase

method. Polypeptides can also be made by chemical synthesis using techniques known in the art.

Polypeptides can also be made by expression systems using recombinant methods. The availability of polynucleotides encoding polypeptides permits the construction of expression vectors encoding intact (i.e., native) polypeptide, functionally equivalent fragments thereof, or recombinant forms. A polynucleotide encoding the desired polypeptide, whether in fused or mature form, and whether or not containing a signal sequence to permit secretion, may be ligated into expression vectors suitable for any convenient host. Both eukaryotic and prokaryotic host systems can be used. The polypeptide is then isolated from lysed cells or from the culture medium and purified to the extent needed for its intended use. Purification or isolation of the polypeptides expressed in host systems can be accomplished by any method known in the art. For example, cDNA encoding a polypeptide intact or a fragment thereof can be operatively linked to a suitable promoter, inserted into an expression vector, and transfected into a suitable host cell. The host cell is then cultured under conditions that allow transcription and translation to occur, and the desired polypeptide is recovered. Other controlling transcription or translation segments, such as signal sequences that direct the polypeptide to a specific cell compartment (i.e., for secretion), can also be used. Examples of prokaryotic host cells are known in the art and include, for example, *E. coli* and *B. subtilis*. Examples of eukaryotic host cells are known in the art and include yeast, avian, insect, plant, nematode, and animal cells such as COS7, HeLa, CHO and other mammalian cells.

When using an expression system to produce mGluR5 polypeptides, it is often preferable to construct a fusion protein that facilitates purification. Examples of components for these fusion proteins include, but are not limited to myc, HA, FLAG, His-6, glutathione S-transferase, maltose binding protein or the Fc portion of immunoglobulin. These methods are known in the art.

Alternatively, in vitro expression systems may also be used to produce mGluR5 polypeptides. A plasmid containing an mGluR5 polynucleotide, under the control of an appropriate promoter, can be transcribed and the resultant RNA translated in vitro through the use of commercially available reagents. Such methods can be used to produce relatively pure, although small amounts of the polypeptide and are known in the art.

Preferably, especially if used for diagnostic purposes, the polypeptides are at least partially purified from other cellular constituents. Preferably, the polypeptides are at least 50% pure. In this context, purity is calculated as a weight percent of the total protein content of the preparation. More preferably, the proteins are 50-75% pure. More highly purified polypeptides may also be obtained and are encompassed by the present invention. For clinical use, the polypeptides are preferably highly purified, at least about 80% pure, and free of pyrogens and other contaminants. Methods of protein purification are known in the art and are not described in detail herein.

10 *Antibodies of the invention*

Also provided by this invention are antibodies capable of specifically binding to mGluR5 polypeptide(s) of this invention. The antibodies can be useful for, for example, for detecting and characterizing mGluR5 polypeptides, as described above. Antibodies of this invention can also be used for purification and/or isolation of polypeptides described herein.

In one embodiment, the invention provides a purified antibody capable of specifically binding to a polypeptide of this invention. As noted in the definition of "antibody" above, this includes fragments of antibodies, such as Fab fragments. In another embodiment, a monoclonal antibody is provided that is capable of specifically binding to a polypeptide of this invention.

Laboratory methods for producing polyclonal antibodies and monoclonal antibodies, as well as deducing their corresponding nucleic acid sequences, are known in the art. For example, see Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York (1988) and Sambrook et al. (1989).

The antibodies of this invention may be polyclonal or monoclonal. Monoclonal antibodies of this invention can be biologically produced by introducing a polypeptide (or fragment of a polypeptide) of this invention into an animal, e.g., mouse or rat. The antibody producing cells in the animal are isolated and fused with myeloma cells or heteromyeloma cells to produce hybrid cells or hybridomas. Accordingly, the invention also includes hybridoma cells producing the monoclonal antibodies of this invention.

Particular isotypes of a monoclonal antibody can be prepared either directly by selecting from the initial fusion, or prepared secondarily, from a parental hybridoma

secreting a monoclonal antibody of different isotype by using the sib selection technique to isolate class switch variants using the procedure described in Steplewski et al. (1985) *Proc. Natl. Acad. Sci.* 82:8653 or Spira et al. (1984) *J. Immunol. Methods* 74:307.

Thus, using the polypeptide(s) of this invention or fragment(s) thereof, and well
5 known methods, one of skill in the art can produce and screen the hybridoma cells and antibodies of this invention for antibodies having the ability to bind polypeptide(s) of this invention.

If a monoclonal antibody being tested binds with an mGluR5 polypeptide(s) of this invention, then the antibody being tested and the antibodies provided by the hybridomas of
10 this invention are equivalent. It is also possible to determine without undue experimentation whether an antibody has the same specificity as a monoclonal antibody of this invention by determining whether the antibody being tested prevents a monoclonal antibody of this invention from binding the polypeptide(s) with which the monoclonal antibody is normally reactive. If the antibody being tested competes with the monoclonal
15 antibody of the invention as shown by a decrease in binding by the monoclonal antibody of this invention, then it is likely that the two antibodies bind to the same or a closely related epitope. Alternatively, one can pre-incubate the monoclonal antibody of this invention with the polypeptide(s) with which it is normally reactive, and determine if the monoclonal antibody being tested is inhibited in its ability to bind the antigen. If the monoclonal
20 antibody being tested is inhibited, then, in all likelihood, it has the same, or a closely related, epitopic specificity as the monoclonal antibody of this invention.

As noted above, this invention also provides biological active fragments of the polyclonal and monoclonal antibodies described above. These antibody fragments retain some ability to selectively bind with its antigen or immunogen. Examples of antibody
25 fragments are known in the art and include, but are not limited to, CDR regions, Fab, Fab', F(ab')₂, F_v, and single chain methods. Methods of making these fragments are known in the art, see for example, Harlow and Lane, (1988).

The antibodies of this invention also can be modified to create chimeric antibodies and humanized antibodies (Oi et al. (1986) *BioTechniques* 4(3):214). Chimeric antibodies
30 are those in which the various domains of the antibodies' heavy and light chains are coded for by DNA from more than one species.

The isolation of other hybridomas secreting monoclonal antibodies with the specificity of the monoclonal antibodies of the invention can also be accomplished by one skilled in the art by producing anti-idiotypic antibodies (Herlyn, et al. (1986) *Science*, 232:100). An anti-idiotypic antibody is an antibody which recognizes unique determinants present on the monoclonal antibody produced by the hybridoma of interest. These determinants are located in the hypervariable region of the antibody. It is this region which binds to a given epitope and, thus, it is responsible for the specificity of the antibody. The anti-idiotypic antibody can be prepared by immunizing an animal with the monoclonal antibody of interest. The animal immunized will recognize and respond to the idiotypic determinants of the immunizing antibody by producing an antibody to these idiotypic determinants. By using the anti-idiotypic antibodies of the second animal, which are specific for the monoclonal antibodies produced by a single hybridoma which was used to immunize the second animal, it is now possible to identify other clones with similar idiotypes as the antibody of the hybridoma used for immunization.

Idiotypic identity between monoclonal antibodies of two hybridomas demonstrates that the two monoclonal antibodies are the same with respect to their recognition of the same epitopic determinant. Thus, by using antibodies to the epitopic determinants on a monoclonal antibody it is possible to identify other hybridomas expressing monoclonal antibodies of the same epitopic specificity.

It is also possible to use the anti-idiotypic technology to produce monoclonal antibodies which mimic an epitope. For example, an anti-idiotypic monoclonal antibody made to a first monoclonal antibody will have a binding domain in the hypervariable region which is the mirror image of the epitope bound by the first monoclonal antibody. Thus, in this instance, the anti-idiotypic monoclonal antibody could be used for immunization for production of these antibodies.

The antibodies of this invention can be linked (i.e., conjugated) to a detectable agent or a hapten. The complex is useful to detect the polypeptide(s) (or polypeptide fragments) to which the antibody specifically binds in a sample, using standard immunochemical techniques such as immunohistochemistry as described by Harlow and Lane (1988). *supra*.

Examples of types of immunoassays which can utilize monoclonal antibodies of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the enzyme linked immunoassay (ELISA)

radioimmunoassay (RIA) and the sandwich (immunometric) assay. Detection of using the monoclonal antibodies of the invention can be done by utilizing immunoassays which are run in either the forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. Those of skill in the art will know, or can readily discern,
5 other immunoassay formats without undue experimentation.

Another technique which may also result in greater sensitivity consists of coupling the antibodies to low molecular weight haptens. These haptens can then be specifically detected by means of a second reaction. For example, it is common to use such haptens as biotin, which reacts avidin, or dinitrophenyl, pyridoxal, and fluorescein, which can react
10 with specific anti-hapten antibodies. See Harlow and Lane (1988) *supra*.

The monoclonal antibodies of the invention can be bound to many different carriers. Thus, this invention also provides compositions containing antibodies and a carrier. Carriers can be active and/or inert. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified
15 celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding monoclonal antibodies, or will be able to ascertain such, using routine experimentation.

There are many different labels and methods of labeling known to those of ordinary
20 skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, and bioluminescent compounds. Those of ordinary skill in the art will know of other suitable labels for binding to the monoclonal antibody, or will be able to ascertain such, using routine experimentation. Furthermore, the binding of these
25 labels to the monoclonal antibody of the invention can be done using standard techniques common to those of ordinary skill in the art.

For purposes of the invention, mGluR5 polypeptides of this invention may be detected by the monoclonal antibodies of the invention by their ability to bind these
antibodies.

30 Compositions containing the antibodies, fragments thereof or cell lines which produce the antibodies, are encompassed by this invention. When these compositions are to be used pharmaceutically, they are combined with a pharmaceutically acceptable

excipient. The compositions may alternatively or additionally contain a physiologically acceptable medium, examples of which have been provided above.

The following examples are provided to illustrate but not limit the invention.

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EXAMPLES

Example 1: Differential effect of CNS depressant propofol on LS and SS mice

Methods

Experimental protocols were approved by our animal care committee. Male LS and
10 SS mice were provided by the Institute for Behavioral Genetics, University of Colorado,
Boulder. They were of the 54th generation (25th generation of selection), were weaned at
25d, and were drug tested at 60-90 d.

Right-sided jugular venous cannulas were implanted under intraperitoneal
pentobarbital (60 mg/kg)-chloral hydrate (125 mg/kg) anesthesia on Day 1. Mice were
15 allowed to rest for 2 d and propofol testing took place on Day 3. Propofol formulated in
Intralipid (10 mg/kg) was obtained from Stuart Chemicals (Wilmington, DE) and
administered intravenously via the jugular cannula. Injected volumes ranged from 30 to 70
μl and were given over 30-45 s by Hamilton syringe. Doses appropriate for loss of righting
reflex ranged from 10 to 35 mg/kg. Saline flush was administered after propofol to bring
20 the total injectate to 100 μl. The loss of righting reflex was immediate in all cases.
Animals were placed on their backs in V-shaped Plexiglas troughs and judged awake upon
regaining the righting reflex (ability to turn over three times in 1 min).

Propofol levels at awakening were determined in brain cortex and plasma samples.
The method of intravenous propofol dosing was simplified by administering 20 mg/kg
25 propofol via the retroorbital venous sinus using a 28-gauge needle and Hamilton syringe.
Injected volumes ranged from 40 to 50 μL and were given over 30 s. At awakening,
cortical brain and body cavity samples were collected. Blood samples were pooled and
centrifuged at 3000g for 15 min; plasma was collected and stored at 4° C until analysis by
gas chromatography. Brain samples were weighed and stored until analysis by gas
30 chromatography. The method of Yu and Liau was used for determination of brain and
plasma levels by gas chromatographic analysis. *J. Chromatog.* (1993) 615:77-81. 2-sec-
Butylphenol was used as an internal standard.

$^{36}\text{Cl}^-$ uptake was performed using freshly prepared mouse brain microsacs as described previously. Allan and Harris (1986) *Life Sci.* 39:2005-2015. Brain cortices were manually homogenized in a glass Teflon homogenizer. Homogenizing solution was 4.5 mL of ice-cold assay buffer containing (mM) NaCl 145, KCl 5, MgCl_2 1, d-glucose 10, 5 CaCl_2 1, and HEPES 10, adjusted to pH 7.5 with Tris base. The homogenate was centrifuged at 900g for 15 min, the supernatant decanted, and the pellet resuspended in 8 mL of assay buffer. It was then centrifuged again at 900g for 15 min. The final pellet was suspended in 7 mL of assay buffer. Protein concentrations were determined by the method of Lowery et al. *J. Biol. Chem.* (1951) 193:265-275.

10 Propofol stimulated $^{36}\text{Cl}^-$ uptake was maximized in the following way: aliquots of microsacs (200 μL) were incubated in a shaking water bath at 34°C for 5 min. $^{36}\text{Cl}^-$ uptake was initiated by addition of 200 μL of a solution containing $^{36}\text{Cl}^-$ (2 $\mu\text{Ci/mL}$). Muscimol (2 μM) and propofol were added in the $^{36}\text{Cl}^-$ solution. $^{36}\text{Cl}^-$ influx was terminated after a 3-s incubation period by addition of 4 mL of ice-cold buffer and rapid filtration under vacuum 15 onto Whatman GF/C glass microfiber filters. Filters were washed with an additional 8 mL of cold buffer. Radioactivity retained on filters was determined by liquid scintillation spectrometry. Control values were obtained using only Intralipid in the preparation. Blank values (no tissue in preparation) were subtracted from all final values. Muscimol-dependent uptake was defined as the amount of $^{36}\text{Cl}^-$ taken up with agonist present in the 20 medium minus the amount of $^{36}\text{Cl}^-$ taken up while agonist was absent (muscimol-independent uptake). Propofol-stimulated uptake was calculated as the uptake in the presence of propofol plus muscimol minus the uptake in the presence of muscimol alone.

Student's unpaired *t*-test was used for comparisons of mean sleep times and drug levels. The $^{36}\text{Cl}^-$ uptake of brain microsacs was compared at several different propofol 25 concentrations using analysis of variance. Statistical significance was set at $P < 0.05$.

Results

Propofol produced dose-dependent increases in sleep times for both LS and SS mice as shown in Figure 1. The LS mice clearly displayed increased sensitivity to propofol, as manifested by longer sleep times, compared with SS mice at every dose. Plasma and 30 cortical brain levels of propofol at awakening were measured and found to be different in the LS and SS mice ($P < 0.0001$). The LS mice had threefold greater plasma levels and twofold greater brain levels than did the SS mice as shown in Table 1. Propofol plasma

levels are expressed in $\mu\text{g/mL}$ plasma \pm SD. Propofol brain levels are expressed in $\mu\text{g/g}$ tissue \pm SD. A propofol dose of 20 mg/kg was given by retroorbital sinus injection. For both comparative measurements, there was a $P < 0.0001$ significant difference between means of LS and SS lines.

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Table 1. Propofol Plasma and Brain Levels at Awakening in Long Sleep (LS) and Short Sleep (SS) Mice

Tissue	LS (n)	SS (n)
Plasma	4.52 ± 0.91 (9)	13.3 ± 1.15 (9)
Brain	8.05 ± 0.9 (13)	16.0 ± 2.4 (15)

The method of propofol dosing was changed from the jugular route (JUG) via the previously implanted catheter to retroorbital venous sinus (ROS) injections using an intravenous dose of 20 mg/kg. We found that the JUG method added several days to a single experiment, produced significant mortality associated with the surgical procedure, and added a prior exposure to the general anesthetics needed to implant the jugular catheter. The ROS method circumvented all these problems. Figure 2 shows the results comparing each of these administrations in SS and LS mice. Although there was a statistically significant difference in sleep times ($P < 0.02$) in the SS line using JUG dosing versus ROS dosing, the difference was only 1.2 min. (Figure 2). A similar but nonsignificant difference of 1.4 min. was observed in the LS line. The difference in sleep time between the SS and LS lines remained very significant ($P < 0.0005$). For both lines, the sleep times were somewhat shorter using JUG dosing as compared to ROS dosing. SS sleep time means \pm SD were 2.24 ± 0.51 min. (JUG) and 3.45 ± 1.02 min. (ROS). LS sleep time means were 4.41 ± 1.29 min. (JUG) and 5.66 ± 1.48 min. (ROS).

We then tested effects of increased propofol on muscimol-stimulated $^{36}\text{Cl}^-$ uptake in LS and SS mice. The results are shown in Figure 3. Propofol produced a concentration-dependent enhancement of muscimol-stimulated $^{36}\text{Cl}^-$ uptake by membrane vesicles (microsacs) prepared from mouse cortical brain. (Figure 3) However, there was no significant difference noted between LS and SS brain preparations in the increase in muscimol-stimulated $^{36}\text{Cl}^-$ uptake at any of the propofol concentrations. Significant

differences in mean $^{36}\text{Cl}^-$ uptake were observed between doses by differed by 100-fold concentrations. Propofol concentrations ranged from 0.50 to 50 $\mu\text{mol/L}$ (corresponding to brain levels of 0.32-31.6 $\mu\text{g/mL}$). Propofol did not affect basal uptake of $^{36}\text{Cl}^-$ except at the highest concentration tested (50 $\mu\text{mol/L}$), which increased basal uptake by about 25%.

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Example 2: Mapping CNS depressant sensitivity

In the absence of a biochemical hypothesis to explain the LS and SS difference, we used a positional cloning strategy to determine the location of the locus (or loci) contributing to this activity.

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Materials and Methods

Origin of Mice

ILS, ISS, and LSXSS RI mice were provided by the Institute for Behavioral Genetics, University of Colorado at Boulder. ILS and ISS have been derived by 20 rounds of brother-sister mating from the LS and SS selected lines; the LSXSS RI set was similarly derived after reciprocal intercrosses between LS and SS, prior to the LS and SS having been inbred. Market et al. (1996); DeFries et al. (1989) *Alcohol Clin Exp Res* 13:196-200. Thus the progenitors of the LSXSS RIs and the F_2 intercross are not the same stock and could have some differences affecting sensitivity to propofol. Animals were bred in a specific-pathogen free facility, weaned at about 25 days and housed 1-4 per cage with like-sex littermates on a 12-hour light/dark cycle. Experimental protocols were approved by animal care and use committees at the University of Colorado at Boulder and University of Colorado Health Sciences Center at Denver. For the LSXSS RIs, between 5 and 15 mice of each sex were tested per strain. For the ILS x ISS F_2 s, 194 mice were tested for LORR and 164 subsequently genotyped. C57BL/6J coisogenic mice having the spontaneous albino mutation c^{2j} were obtained from Jackson Laboratory, Bar Harbor, Maine.

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Phenotypic Assessments

Propofol (10 mg/ml in Intralipid, Stuart Chemicals) injections were performed when mice were 60 to 94 days of age. Dosages of 20 mg/kg (volumes ranging from 30-70 μl) were administered by injection into the retroorbital venous sinus using a 26-gauge, 3/8-inch needle and Hamilton syringe. Sensitivity to propofol was assessed using the duration of loss of righting reflex (LORR). Simpson et al. (1996). Upon injection, mice

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immediately lost consciousness and were placed on their back in V-shaped Plexiglass troughs until recovery of righting reflex. Animals were judged to have regained the righting reflex when they turned over three times in less than a minute. Temperature was controlled at 24-26°C. Full recovery was quick after regaining consciousness, as typically
 5 observed in recovery from propofol anesthesia in humans; after the first righting, there was almost no delay in the mouse's ability to right a second and third time. The RI and F₂ mice were naive to any previous injections and were injected only once with propofol. LORRs of the (ISS *c/c* x C57BL/6 *c²/C*) F₁ mice represent the mean of two propofol injections spaced seven days apart whose means were not significantly different ($p = 0.36$, ANOVA
 10 two-tailed significance). The brain levels of propofol at awakening (BLA) for the RIs were determined in pooled samples of brain cortex using gas chromatography, as previously described by Simpson and Blednov (1996).

Marker Assessments

DNA was extracted and purified from spleen, kidney, or liver using a Super-Quik
 15 Gene DNA Isolation Kit (AGTC Research). The *Mit* SSLP primers were obtained from Research Genetics and the PCR was carried out as previously described. Markel et al. (1994) *Mam. Genome* 5:199-202. Coat color was scored by eye on living mice; pigmented RI mice were homozygous *C/C* and albino RI mice were homozygous *c/c*. Jackson et al. (1984) *Proc. Natl. Acad. Sci.* 87:7010-14. (This locus was renamed *Tyr* because it encodes
 20 tyrosinase, an enzyme responsible for pigmentation in mammals. See Beerman et al. (1990) *EMBO J.* 9:2819-2826; Yokoyama et al. (1990) *Nucl. Acids Res.* 18:7293-7298.)

Statistical Analyses

Broad-sense heritability involves genetic effects arising from dominant, additive
 25 and between-gene interactions while narrow-sense heritability involves only additive effects; thus broad-sense heritability is more inclusive and should be a larger fraction than narrow sense. Narrow-sense heritability in the RIs was estimated as $100\% \times 0.5 \sigma_B^2 / (\sigma_B^2 + 0.5\sigma_W^2)$, in which σ_B^2 is the between-strain component of variance and σ_W^2 is the within-strain component of variance. Falconer (1981) *Quantitative Genetics*, Ronald, New York. Broad sense heritability was estimated from the ILS by ISS F₂s as $100\% \times (V_{F2} - V_{F1}) / V_{F2}$,
 30 where V_{F2} is the variance of the F₂ population and V_{F1} represents an average variance calculated from the F₁, ILS, and ISS variances. The QTL maps in Figures 2 and 3 were anchored using map positions from the Mouse Genome Database (MGD) map

(<http://www.informatics.jax.org/mgd.html>) of 44.0 for *c* and D7Mit31, whereas the positions of other SSLP markers (Copeland et al. (1993) *Science* 262:57-66; Dietrich et al. (1994) *Nature Genet.* 7:220-225; Dietrich et al. (1996) *Nature* 380:149-152) relative to these were based on the Whitehead Institute/MIT Center for Genome Research (WI/MIT CGR) map (<http://www.genome.wi.mit.edu>), which is the more recently updated and more accurate map regarding the relative position of SSLP markers in this region. Rikke et al. (1997) *Genetics* 147:787-799. The RI map was constructed using an interval-mapping procedure previously described by Markel et al. (1996) *Behav. Genet.* 26:447-458. The F₂ linkage map was constructed using Mapmaker 3.0 and QTL mapping was done using Mapmaker/QTL Version 1.1.

Results

Heritability of Propofol Sensitivity

The results are shown in Table 2. For each population or cross, the female parent is listed first. There was a significant difference in sensitivity to propofol between the LS and SS lines ($p < .001$; Table 2, lines 1, 2), as described in Example 1 (and previously reported by Simpson et al. (1996)); their inbred derivatives, ILS (LORR of 7.4 minutes) and ISS (LORR of 3.8 minutes), also are differentially sensitive to propofol ($p < .001$; Table 2, lines 3, 4). The ILS and ISS strains were also significantly different from the two F₁ reciprocal populations with respect to propofol sensitivity. The F₁ showed no statistically significant differences (Table 2, lines 5, 6; $p = 0.08$, one-tailed) resulting from the maternal parent, thus indicating a lack of maternal effects for sensitivity to propofol. The mean LORR of the F₁ was almost intermediate between the two parents but showed slight dominance of the LS allele.

Table 2. Duration of loss of righting reflex (LORR) after propofol injection in various mouse populations.

Population	Trait	Total N	Mean \pm SEM (minutes)	Variance (minutes)
1. LS	LORR	19	5.7 \pm 0.3	1.8
2. SS	LORR	16	3.5 \pm 0.2	0.88
3. ILS	LORR	19	7.4 \pm 0.4	2.7
4. ISS	LORR	37	3.8 \pm 0.2	2.2
5. F ₁ , ILS \times ISS	LORR	17	6.5 \pm 0.5	3.8
6. F ₁ , ISS \times ILS	LORR	8	5.3 \pm 0.7	3.4
7 F ₁ combined	LORR	25	6.1 \pm 0.4	3.7
F ₂ ILS/ISS \times ILS/ISS	LORR			
8. pigmented		32	6.7 \pm 0.5	9.6
9. albino		18	6.1 \pm 0.6	6.7
F ₂ ILS/ISS \times ILS/ISS	LORR			
10. pigmented		35	6.8 \pm 0.4	7.0
11. albino		8	4.3 \pm 0.8	5.3
F ₂ ISS/ILS \times ILS/ISS	LORR			
12. pigmented		36	6.7 \pm 0.3	2.9
13. albino		18	6.2 \pm 0.7	8.0
F ₂ ISS/ILS \times ISS/ILS	LORR			
14. pigmented		32	7.1 \pm 0.6	9.8
15. albino		15	5.7 \pm 0.6	4.6
F ₂ All	LORR	194	6.5 \pm 0.2	6.9
16. pigmented		135	6.8 \pm 0.2	7.1
17. albino		59	5.8 \pm 0.3	6.5
18. N ₂ ILS \times ILS/ISS	LORR	27	6.6 \pm 0.4	4.9
19. N ₂ ISS/ILS \times ILS	LORR	28	6.0 \pm 0.5	6.1
N ₂ ISS \times ILS/ISS				
20. pigmented	LORR	19	6.2 \pm 0.6	7.8
21. albino	LORR	6	5.2 \pm 0.6	2.2
22. total	LORR	25	6.0 \pm 0.5	6.5
N ₂ ILS/ISS \times ISS				
23. pigmented	LORR	15	7.6 \pm 0.6	4.7
24. albino	LORR	14	6.7 \pm 0.5	3.2
25. total	LORR	29	7.1 \pm 0.4	4.1
			(μ g/g brain)	
LS	BLA	11	8.2 \pm 0.2	0.81
SS	BLA	10	16.1 \pm 0.6	5.8

Estimates of heritability show what fraction of the variation among individuals is due to genetic effects. Falconer (1981). We have estimated these heritabilities for differential sensitivity to propofol using two distinct approaches. First, an estimate of broad-sense heritability (h^2) can be obtained by assessing the variation in length of LORR after propofol injection in the inbred parental strains (ILS and ISS) and in the F₂ reciprocal

cross (Table 2). An F₂ population derived from an ILS by ISS and the reciprocal intercross had a mean not different from the F₁. Comparing the F₂ variance with the average variance of the F₁ and the two parental strains we derived an estimate of broad-sense heritability of 55%. Falconer (1981).

5 A second estimate can be obtained by dividing 1/2 the variance among the 24 RI strain means by 1/2 the inter-strain variance plus the variance within the 24 RIs. Falconer (1981). Using this method, which estimates narrow-sense heritability, we found that about 32% of the variance is genetic. Broad-sense heritability includes estimates of dominance and interactions among loci while narrow-sense heritability only estimates additive effects; 10 these results thus suggest that there may be a substantial genetic component resulting from epistatic (between different genes) interactions, but both methods suggest a large overall genetic component. An estimate of BLA heritability could not be obtained because of an inability to collect BLA on individual mice due to the limited sensitivity of the assay.

Location of a QTL for Propofol Sensitivity

15 The LS and SS selected lines are almost completely differentiated at the albino locus (*Tyr*) with all SS mice being albino and most of the LS mice being pigmented. Surprisingly, when we examined the LSXSS RIs we found that the eleven albino RI strains averaged significantly shorter LORRs in response to propofol than did the 13 pigmented RI strains ($p < .0002$), with no difference between males and females as shown in Fig. 4A. The 20 difference in LORR between males and females was not significant; whereas, the LORR difference between pigmented (black bars, $4.8 \pm \text{SEM}$) and albino RI strains (white bars, 9.3 SEM) was highly significant for both males and females ($p < 0.0001$, Student's *t* test two-tailed significance). "n" refers to the number of RI strains. The LORR for each strain was determined from the mean of 5-15 animals of each sex, and the BLA for each strain 25 was determined from the mean of at least 3 animals. The difference in propofol BLA between pigmented ($8.4 \pm 1.2 \mu\text{g/gm}$) and albino ($17.2 \pm 1.9 \mu\text{g/gm}$) strains is highly significant ($p < 0.0001$, Student's *t* test two-tailed significance). The pigmented RI strains (black bars) used for propofol BLA determination were 2, 3, 6, 7, 8, 9, 17, 30 and 36; and the albino RI strains (white bars) used were 4, 10, 18, 20, 23, 25, and 33. Male and female 30 data are combined for propofol BLA. The correlation between propofol BLA and LORR for the 16 RI strains for which both measures could be obtained was -0.85 ($p < 0.001$). RI 32 was excluded from this analysis because it was still segregating for the albino mutation.

The albino strains also had very significantly higher BLA ($p < .0001$; Fig 1A), suggesting the difference in LORR is due to differential CNS sensitivity and not pharmacokinetic differences. The correlation coefficient between BLA and LORR was 0.85 and highly significant ($p < 0.001$). The LORRs of the albino strains were non-overlapping with the
5 LORRs of the pigmented strains, demonstrating a major effect of a QTL linked to or at the *Tyr* locus.

This QTL was mapped using the regression approach described by Haley and Knott, which we previously used in mapping QTLs for alcohol sensitivity. We assessed the genotypes of seven additional SSLP markers spanning 50 centiMorgan (cM) of
10 chromosome 7, obtaining LOD scores of 19 and 21 for males and females, respectively. The combined LOD score for males and females was about 28 (Fig. 5), far exceeding the value of 3.3 recommended by Lander and Kruglyak for significant linkage in a whole-genome scan. This assignment was highly specific and positioned the QTL to within 2.5 cM of *Tyr* with 2-LOD support, indicating the probability of the QTL being within this
15 region is greater than 0.99. The peak LOD score was at the *Tyr* gene itself. This QTL, which we have called *Lorpl* (*Loss Of Righting* due to Propofol), explained about 80% of the genetic variance between LS-like and SS-like RI strains, indicating this is the major gene specifying differential propofol sensitivity between LS and SS. BLA also mapped to this region, peaking at the *Tyr* locus, although with significantly reduced LOD score, not
20 passing the suggested level of 3.4 needed to assure that the QTL is real. With respect to Fig. 7, ISS mice have the classical albino mutation, whereas the c^{2j} albino mutation arose spontaneously in C57BL/6 at Jackson Laboratory. Therefore, F_1 mice are identical at all loci except the albino locus, pigmented mice (black bars) are c/C and albino mice (white bars) are c/c^{2j} . LORRs of females are significantly shorter than those of males by 1.3
25 minutes ($p = 0.002$, ANOVA two-tailed significance). However, LORRs of albinos are not significantly shorter than those of pigmented mice ($p = 0.35$, ANOVA one-tailed significance).

Confirmation of Linkage

Although confirmation of linkage after such an astronomical LOD score is not
30 needed, the map location of *Lorpl* was confirmed independently using a panel of 164 F_2 mice derived from intercrosses of ILS and ISS. These F_2 s were genotyped with 16 SSLP markers from murine chromosome 7 that included all of the informative SSLP markers

flanking *Tyr* for about 10 cM (WI/MIT CGR map and Rikke et al., submitted). A peak LOD score of 3.9 was obtained between markers *D7Mit31*, which is within *Tyr*, and *D7Mit123*, which is less than 2 cM distal to *Tyr* (Fig 6). We found that F₂ homozygotes for the SS allele of *D7Mit123*, slept 5.7 minutes (SEM = 0.3), heterozygotes slept 6.5 minutes (SEM = 0.3), and LS homozygotes slept 8.0 minutes (SEM = 0.4). The 2.3 minute difference in propofol-induced LORR between mice homozygous for the S allele at *D7Mit123* and those homozygous for the L allele was consistent with the 2.2 minute difference previously observed between LS and SS, suggesting that all of the genetic difference between LS and SS for propofol sensitivity has been captured in the ILS and ISS strains and that most or all of the genetic difference resides in *Lorpl*.

Location of a QTL for Ethanol, Enflurane, Isoflurane and Etomidate

The QTL location experiments described above were repeated for CNS depressants ethanol (4.1 g/kg), isoflurane (2.0 g/kg), enflurane (5.0 g/kg) and etomidate (20 mg/kg). The behavioral measure of sensitivity was LORR (as was used in the propofol studies, above). The results (including those for propofol) are shown in Figs. 8-10. The peak LOD scores are at or very close to *Tyr* for each CNS depressant, just as with propofol. However, the 2-LOD support interval for each is much broader than 2.5 cM as reported for propofol (on the order of 25-30 cM). The results indicate that the genetic locus associated with differential CNS sensitivity is linked to the *Tyr* locus.

Example 3: A 250 kb fragment in yeast artificial chromosome YRT2 confers increased sensitivity to propofol

Transgenic mice harboring YAC YRT2 (covering the mouse tyrosinase locus as isolated from a YAC library of C3H mouse DNA) were kindly provided by Schütz. Schedl et al. (1993) *Nature* 362:258-261. YRT2, the resulting 250 kb construct, contains 80 kb of the tyrosinase coding region (Ruppert, S. et al. *EMBO J.* 7:2715-2722, (1988)), 155 kb of upstream sequences and 15 kb of vector DNA (Fig. 11).

The transgenic mice were originally created in outbred albino mouse strain NMRI. These mice were crossed with albino ISS strain. Progeny from crossing two different strains were denoted F₁S. Thus, F₁S mice were 50% NMRI and 50% ISS. The F₁S mice

were then backcrossed with ISS, and these progeny were denoted N₂S (which were 25% NMRI and 75% ISS). For both F₁S and N₂S, mice having the transgene were pigmented, whereas non-transgenic littermates were albinos.

5 We tested three different YRT2 lines for these experiments. Transgenes insert at random sites in the genome; therefore, it was presumed that each line had its transgenic insert at a different site. Transgenes also tend to integrate as tandemly repeated copies of the original transgenic insert. Schedl et al. determined that one of the transgenic lines had one copy of the transgenic region (denoted Tg1), another line had two copies (Tg2), and another line had eight copies (Tg8). More copies of the transgene generally implies greater
10 expression levels of the transgene, which could explain the greater difference seen with the Tg8 mice. However, expression levels may also depend on where in the genome the DNA has inserted.

Sleep times of transgenic mice were compared to sleep times of non-transgenic littermates. All mice (60 to 90 days old) received 20 mg/kg propofol. The results are
15 shown in Fig. 12. N indicates the number of mice tested. For each comparison, the difference was statistically significant. The transgenic mice showed longer sleep time (i.e., increased sensitivity) than non-transgenic mice. In the first group, transgenic mice slept an average of 0.7 minutes longer than non-transgenic mice (p= 0.01). In the second group, transgenic mice slept an average of 0.6 minutes longer than non-transgenic mice (p=0.056).
20 In the third group, transgenic mice slept an average of 1.5 minutes longer than non-transgenic mice (p=0.03). Sleep-time sensitivity was independent of transgene insertion site, indicating that the locus *Lorpl* is within the transgenic region, i.e., within the 250 kb YAC insert.

25 **Example 4: Analysis of sequences on YRT2,
including mGluR5 sequences**

We analyzed sequences contained in YRT2 in an effort to determine which sequences of the 250 kb insert may be particularly associated with differential CNS depressant activity. To accomplish this, we first isolated a BAC clone as follows. A
30 mouse BAC genomic library was obtained from a commercial source (Research Genetics). The library was screened by PCR using primer pairs corresponding to known genetic markers that were known to reside on the 250 kb insert of YRT2. A positive BAC clone

was identified.. To convert the BAC into a small insert sequence-ready library, we followed the scheme depicted in Fig. 13. BAC DNA was sheared using a sonicator and fragments of about 1.5 kb were size selected on agarose gels and isolated. The size-selected fragments were blunt end ligated into a pBLUESCRIPT™ vector which had been
5 SmaI linearized and dephosphorylated. The ligated vectors containing the size-selected fragments were electroporated into XLI-BLUE™ electrocompetent cells. Blue colonies were picked, and the inserts sequenced using fluorescence (Applied Biosystems ABI373A).

Once a sequence had been obtained, it was compared to sequences in GenBank
10 using a BLAST searches. The comparison revealed that some sequences were homologous to human and rat mGluR5, with rat mGluR5 exhibiting the highest homology (SEQ ID NOS:5 and 6). A more extensive sequence of the mGluR5 gene was obtained by sequencing this region using DNA amplified from total brain cDNA from LS and SS mice.

The polynucleotide sequence of a coding region of mouse mGluR5 is shown in
15 SEQ ID NO:1. A polypeptide sequence for mouse mGluR5 is shown in SEQ ID NO:2. A comparison of over 90% of the amino acid sequence for mGluR5 in LS and SS mice showed no differences in either the amino acid or polynucleotide sequences.

20

Example 5: Glutamate receptor modulation affects propofol sensitivity

LS and SS mice receiving propofol and agonist ACPD

25 LS and SS mice were tested for the effect of ACPD, a group I metabotropic agonist, on propofol sleep time. Five LS (sensitive) mice (60 to 90 days old) and three SS (resistant) mice (60 to 90 days old) were used for this experiment. Intracerebroventricular catheters were implanted under pentobarbital (PB, 80 mg/kg) chloral hydrate (CH, 120 mg/kg) anesthesia, administered by intraperitoneal injection. After a 3 day recovery, propofol was
30 administered at 20 mg/kg by retroorbital sinus injection. 5 µl of agonist ACPD (Tocris-Cookson) was administered into the lateral ventricles via the implanted catheter. After 30 minutes, the propofol sleep time was assessed. Three LS and three SS control mice

received 5 μ l saline instead of agonist. Sleep time, measured as LORR, was recorded for test mice and controls. After sleep times were measured, the mice were sacrificed and the brains dissected to confirm the proper placement of the ventricular catheter. In all animals, the catheter was in proper position.

5 The results are tabulated in Table 4. SS (resistant) mice receiving agonist displayed a greater degree of increased resistance (when compared to SS controls) than LS (sensitive) mice receiving agonist. SS mice slept an average of 2.70 minutes shorter with agonist than without agonist, while LS mice slept an average of 0.67 minutes shorter with agonist than without agonist. These results are consistent with the hypothesis that the mGluR system
10 (particularly those in Class I) is more sensitive to stimulation by agonist in SS (resistant) mice than LS mice, and thus may at least partially account for the difference in responsiveness between LS and SS mice.

Transgenic mice receiving agonist

Transgenic mice as described in Example 3 received approximately 40 μ l propofol
15 (20 mg/ml) and 5 μ l agonist ACPD (62.5 pmol/5 μ l). Sleep times were measured as described. The results are shown in Table 3. Transgenic mice slept an average of 3.97 minutes, while control (non-transgenic littermates) mice slept an average of 0.83 minutes. The average (mean) difference was 3.14 minutes, with a p value of 0.028523.

20 **Example 6: Screening Using YRT2 Sequences**

The following example describes cell-based and in vivo screening assays which use mGluR5 polynucleotide sequences found within the YRT2 sequence, but any analogous sequences (e.g., transcriptional control regions, polypeptide-encoding sequences) found within the YRT2 sequence may also be used in the following screens and assays.

25 In an example to identify such agents, an mGluR5-green fluorescent protein (GFP) hybrid gene is assembled in which the expression of GFP is under the control of the mGluR5 transcriptional control region and this hybrid gene is stably introduced into a mammalian cell line (e.g., CHO cells or a cell line with neuronal characteristics).

To screen for agents that modulate the expression of the mGluR5 gene (and may
30 thus effect CNS depressant activity), cells that carry the mGluR5-GFP hybrid gene are grown in standard media and an agent to be tested is added (control cells receive no agent). After a suitable time, the cell suspensions are checked for GFP fluorescence. These assays

may be rapidly and conveniently performed in microtiter plates, using a small amount of media and agent to be tested in each well with the cells. Agents are identified by their ability to increase or decrease the amount of GFP fluorescence relative to that of the control cells. A relative increase in GFP fluorescence in cells grown in the presence of the agent may indicate an activation of expression driven from the mGluR5 regulatory elements by the agent. A relative decrease in GFP fluorescence in cells grown in the presence of the agent may indicate a suppression of expression from said regulatory elements. This mGluR5-GFP hybrid gene assay is performed in the presence of a CNS depressant to screen for agents which may modulate the depressant activity on mGluR5 expression, if any.

In another example to identify agents which exhibit CNS depressant activity or which modulate the activity of a CNS depressant, sequences encoding an mGluR5 polypeptide are expressed in cells (e.g., CHO cells or a neuronal cell line) under control of a promoter active in the particular cell type chosen. The promoter may be the endogenous mGluR5 promoter or a heterologous (including an inducible) promoter.

To screen for agents that modulate the activity of the mGluR5 polypeptide (and may thus effect CNS depressant activity), cells that express the mGluR5 polypeptide are grown in appropriate media and an agent to be tested is added (control cells receive no agent). After a suitable time, mGluR5 activity in the cells is measured through, for example, the determination of intracellular calcium release (using, for example, a fluorescent calcium indicator such as fura-2). Agents are identified by their ability to increase or decrease the activity of mGluR5 relative to that in the control cells. This mGluR5 activity assay is performed in the presence of a CNS depressant to screen for agents which may modulate the activity of the depressant on mGluR5 activity, if any.

After the identification of agents using cells in culture, the agents are tested in vivo for their effect on CNS depressant activity. Transgenic mice harboring the YRT2 sequences allow the agents to be tested in the context of the CNS and allow the use of assays for CNS depressant sensitivity as described above (e.g., LORR). The transgenic mice for this assay could be those as described in Example 3. As indicated in Example 3, the CNS depressant sensitivity of the transgenic mice appears to be of an intermediate level between that of SS mice and that of LS mice at which an agent can be tested for its ability to increase or decrease the CNS depressant sensitivity. Control over the heterozygosity or

the copy number of transgenic YRT2 sequences may provide an animal with a suitable level of CNS depressant sensitivity of this screen.

5 The agent is administered to animals with or without a CNS depressant (e.g., propofol). Determination of the CNS depressant activity of the agent is made using the standard LORR assessment protocols described in previous Examples.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent to those skilled in the art that certain changes and modifications can be practiced. Therefore, the description and examples should not be construed as limiting the scope of the invention,
10 which is delineated by the appended claims.

First Set of Transgenics Used in Study

Line	Cage	DOB	Mou	Color	weight	DOI for S	Inj PB	Inj CH	DOI for ST	Weight	Age	Δt^1	Agonist	T Inj Ag	Δt^2	Inj Prop (μ l)	T out	T Up	ST	brain Vial	Hit ICV
Tg8xISS	1	7230	1M	Brown	20.1	7318	0.20	0.20	7322	19.4	92	4	5 μ l	0.00	41.50	38.8	41.50	45.50	4.00	1	Y
Tg8xISS	2	7228	1M	brown	20.8	7318	0.21	0.21	7322	19.9	94	4	5 μ l	51.00	42.50	39.8	93.50	96.00	2.50	3	Y
Tg8xISS	3	7250	2M	brown	22.3	7318	0.22	0.22	7322	22.2	74	4	5 μ l	62.50	37.83	44.4	100.33	105.75	5.42	5	Y
																		<u>Mean</u>	<u>SD</u>		
																		3.97	1.46		
Control	1	7230	3F	white	19.0	7318	0.19	0.19	7322	18.7	92	4	5 μ l	12.00	?	37.4	NS	NS	0.00	2	Y
Control	2	7228	2F	white	18.3	7318	0.18	0.18	7322	20.8	94	4	5 μ l	55.00	39.83	41.6	94.83	97.33	2.50	4	Y
Control	3	7250	1F	white	17.9	7318	0.18	0.18	7322	19.1	72	4	5 μ l	59.50	?	38.2	NS	NS	0.00	CHO	X
																		<u>Mean</u>	<u>SD</u>		
																		0.83	1.44		
																		A Mean			3.14
																		P-Value			0.028523

DOB = Date of Birth
 DOI = Date of Injection
 S = Surgery
 PB = Pentobarbital concentration
 CH = Chloral Hydrate concentration
 ST = Sleep Time, Time to Unconsciousness
 Δt^1 = number of days in which the animal was observed
 Agonist = ACPD 62.5 mg/kg
 Prop = Propofol, concentration
 Δt^2 = number of minutes
 CFO = Cannula Fell out

CLAIMS

What is claimed is:

1. A method of identifying an agent which may exhibit CNS depressant activity, comprising the steps of:
 - (a) introducing a polynucleotide comprising a mouse polynucleotide sequence associated with CNS depressant sensitivity into a suitable host cell, wherein the mouse polynucleotide sequence corresponds to a polynucleotide sequence of yeast artificial chromosome YRT2;
 - (b) contacting host cell of step (a) with at least one agent to be tested;
 - (c) analyzing at least one characteristic associated with expression of the mouse YRT2 polynucleotide, wherein an agent is identified by its ability to modulate expression of the mouse YRT2 polynucleotide.
2. The method of claim 1, wherein the mouse polynucleotide is contained in an mGluR5 gene.
3. The method of claim 2, wherein the mouse polynucleotide is a control region of the mGluR5 gene.
4. A method of classifying a CNS depressant, comprising the steps of:
 - (a) introducing a polynucleotide comprising a mouse polynucleotide sequence associated with CNS depressant sensitivity into a suitable host cell, wherein the mouse polynucleotide sequence corresponds to a polynucleotide sequence of yeast artificial chromosome YRT2;
 - (b) contacting host cell of step (a) with at least one agent to be tested;
 - (c) analyzing at least one characteristic associated with expression of the mouse YRT2 polynucleotide, wherein an agent is identified by its ability to modulate expression of the mouse YRT2 polynucleotide, and wherein a CNS depressant which modulates expression may fall within a class of CNS depressants which displays differential reactivity in LS and SS mice.
5. The method of claim 4, wherein the mouse polynucleotide is contained in mGluR5 gene.
6. The method of claim 6, wherein the mouse polynucleotide is a control region of the mGluR5 gene.
7. A method of identifying an agent which may modulate CNS depressant sensitivity in an individual, said method comprising:
 - (a) introducing a polynucleotide comprising a mouse polynucleotide sequence associated with CNS depressant sensitivity into a suitable host cell, wherein the mouse polynucleotide sequence corresponds to a polynucleotide sequence of yeast artificial chromosome YRT2;
 - (b) contacting host cell of (a) with at least one agent to be tested;

- (c) analyze at least one characteristic associated with expression of the mouse YRT2 polynucleotide, wherein an agent is identified by its ability to modulate expression of the mouse YRT2 polynucleotide.
8. The method of claim 7, wherein the mouse polynucleotide is contained in an mGluR5 gene.
9. The method of claim 8, wherein the mouse polynucleotide is a control region of the mGluR5 gene.
10. A method of identifying an agent which may exhibit CNS depressant activity, comprising the steps of:
- (a) introducing a polynucleotide comprising a metabotropic glutamine receptor polynucleotide sequence into a suitable host cell;
 - (b) contacting host cell of step (a) with at least one agent to be tested;
 - (c) analyze at least one characteristic associated with expression of the mammalian metabotropic glutamate receptor polynucleotide, wherein an agent is identified by its ability to modulate expression of the mammalian metabotropic glutamate receptor polynucleotide.
11. The method of claim 10, wherein the metabotropic glutamate receptor polynucleotide is mammalian.
12. The method of claim 11, wherein the mammalian metabotropic glutamate receptor polynucleotide is human.
13. The method of claim 11, wherein the mammalian metabotropic glutamate receptor is mGluR5.
14. The method of claim 11, wherein the mammalian metabotropic glutamate receptor is mGluR1.
15. A method of classifying a CNS depressant, comprising the steps of:
- (a) introducing a polynucleotide comprising a metabotropic glutamate receptor polynucleotide sequence into a suitable host cell;
 - (b) contacting host cell of step (a) with at least one agent to be tested;
 - (c) analyzing at least one characteristic associated with expression of the metabotropic glutamate receptor polynucleotide, wherein an agent is identified by its ability to modulate expression of the metabotropic glutamate receptor polynucleotide sequence, and wherein a CNS depressant which modulates the metabotropic glutamate receptor polynucleotide expression may fall within a class of CNS depressants which displays differential reactivity in LS and SS mice.
16. The method of claim 15, wherein the metabotropic glutamate receptor polynucleotide is mammalian.

17. The method of claim 16, wherein the mammalian metabotropic glutamate receptor polynucleotide is human.
18. The method of claim 17, wherein the human metabotropic glutamate receptor is mGluR5.
19. The method of claim 16, wherein the human metabotropic glutamate receptor is mGluR1.
20. An isolated polynucleotide comprising a polynucleotide encoding a mouse mGluR5 polypeptide, wherein the mouse mGluR5 polypeptide is at least about 10 contiguous amino acids of SEQ ID NO:2 and exhibits mGluR5 activity, and wherein the at least about 10 contiguous amino acids are not depicted within SEQ ID NO:6 or SEQ ID NO:7.
21. An isolated polynucleotide comprising a polynucleotide of at least about 25 contiguous nucleotides of SEQ ID NO:1, wherein the at least about 25 contiguous nucleotides are not depicted in SEQ NO: 3 or SEQ ID NO:5. --
22. An isolated polynucleotide comprising a region of at least about 25 contiguous nucleic acids of SEQ ID NO:1, said region having at least about 97% sequence identity to a sequence in SEQ ID NO:1.
23. A cloning vector comprising the polynucleotide of claim 20.
24. A cloning vector comprising the polynucleotide of claim 21.
25. A cloning vector comprising the polynucleotide of claim 22.
26. An expression vector comprising the polynucleotide of claim 20.
27. An expression vector comprising the polynucleotide of claim 21.
28. An expression vector comprising the polynucleotide of claim 22.
29. A host cell comprising the polynucleotide of claim 20.
30. A host cell comprising the polynucleotide of claim 21.
31. A host cell comprising the polynucleotide of claim 22.
32. The host cell of claim 29, wherein the host cell is mammalian.
33. The host cell of claim 30, wherein the host cell is mammalian.
34. The host cell of claim 31, wherein the host cell is mammalian.
35. An isolated polypeptide comprising at least about 5 contiguous amino acids of the sequence of SEQ ID NO:2, and wherein the at least about 5 contiguous amino acids are not depicted in SEQ ID NO:6 or SEQ ID NO:7.
36. The polypeptide of claim 35, wherein the polypeptide comprises the sequence of SEQ ID NO:2.
37. A purified antibody capable of specifically binding to a polypeptide of claim 35.
38. A monoclonal antibody capable of specifically binding to a polypeptide of claim 35.

1 / 30

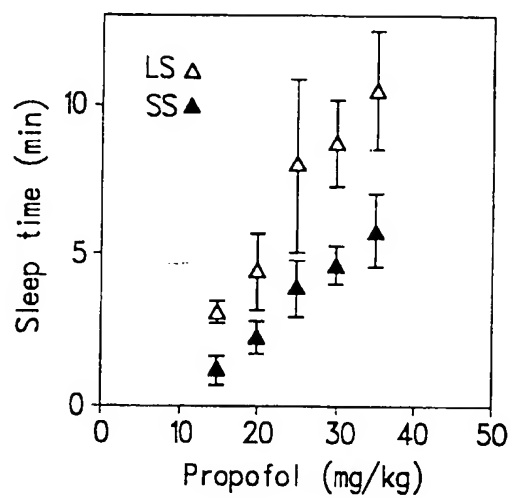


FIG. 1

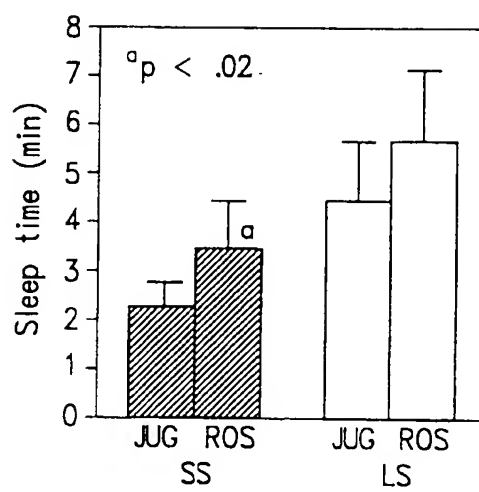
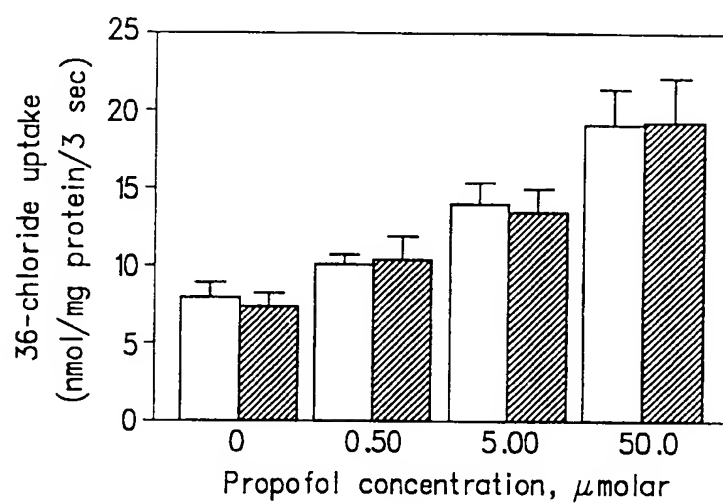
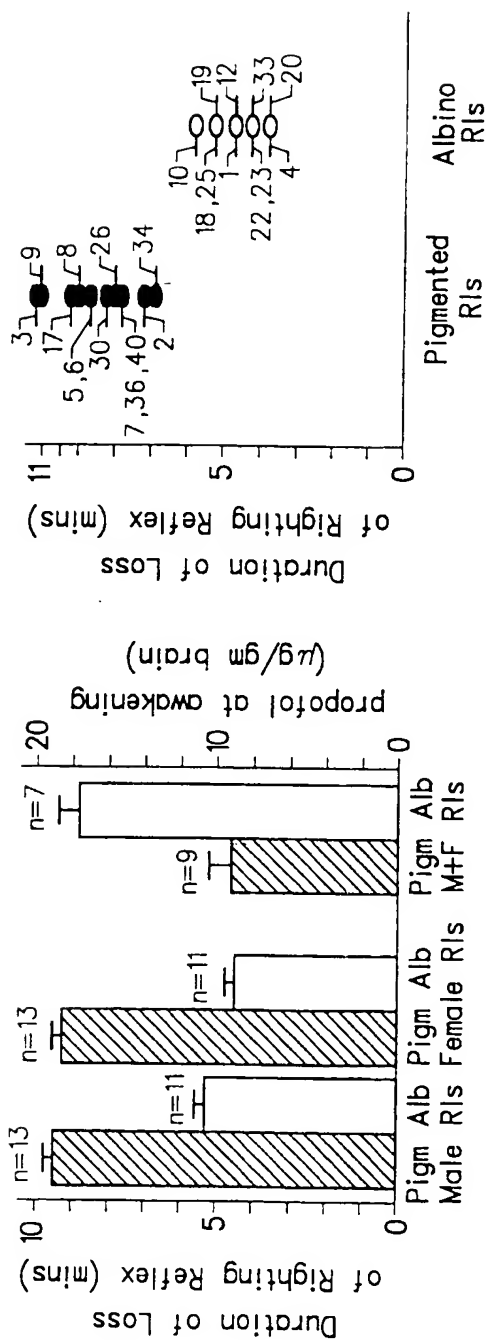


FIG. 2

*FIG. 3*



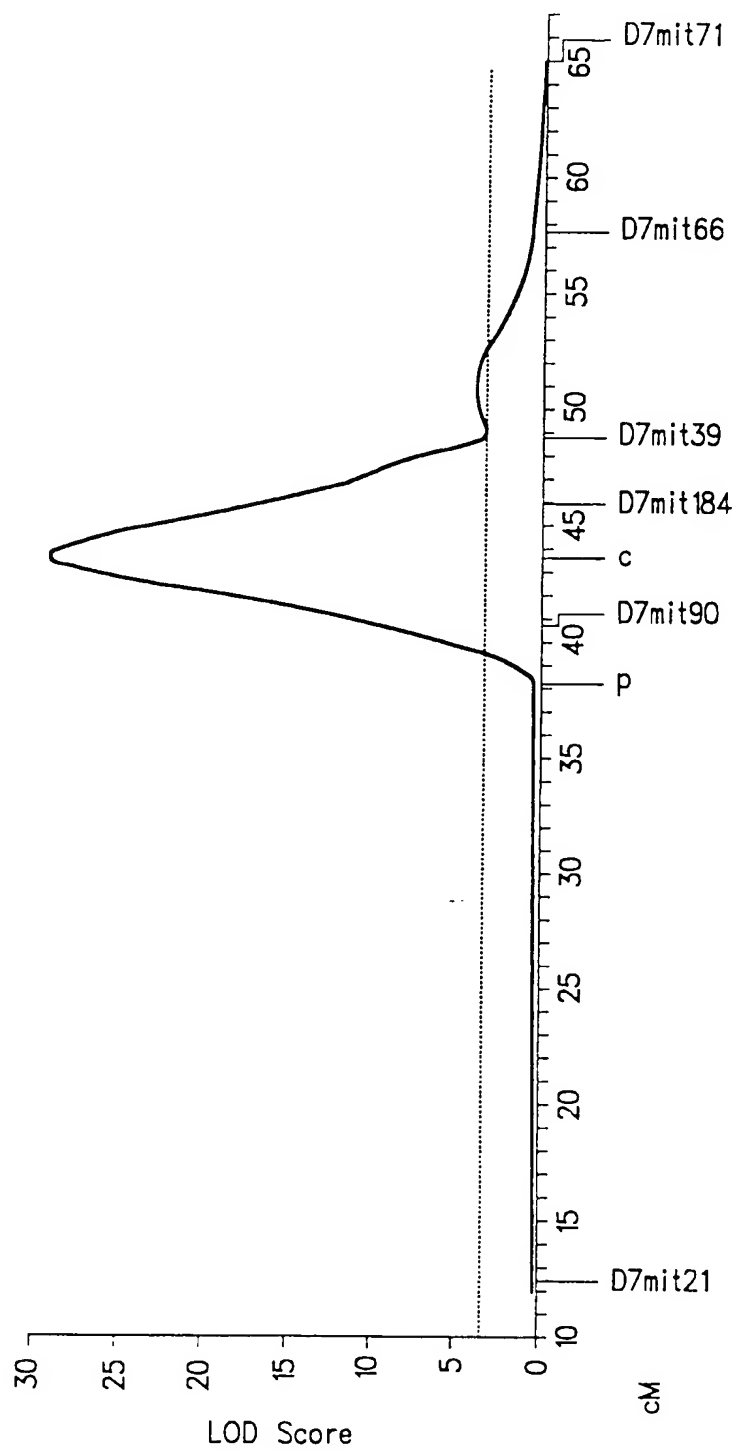


FIG. 5

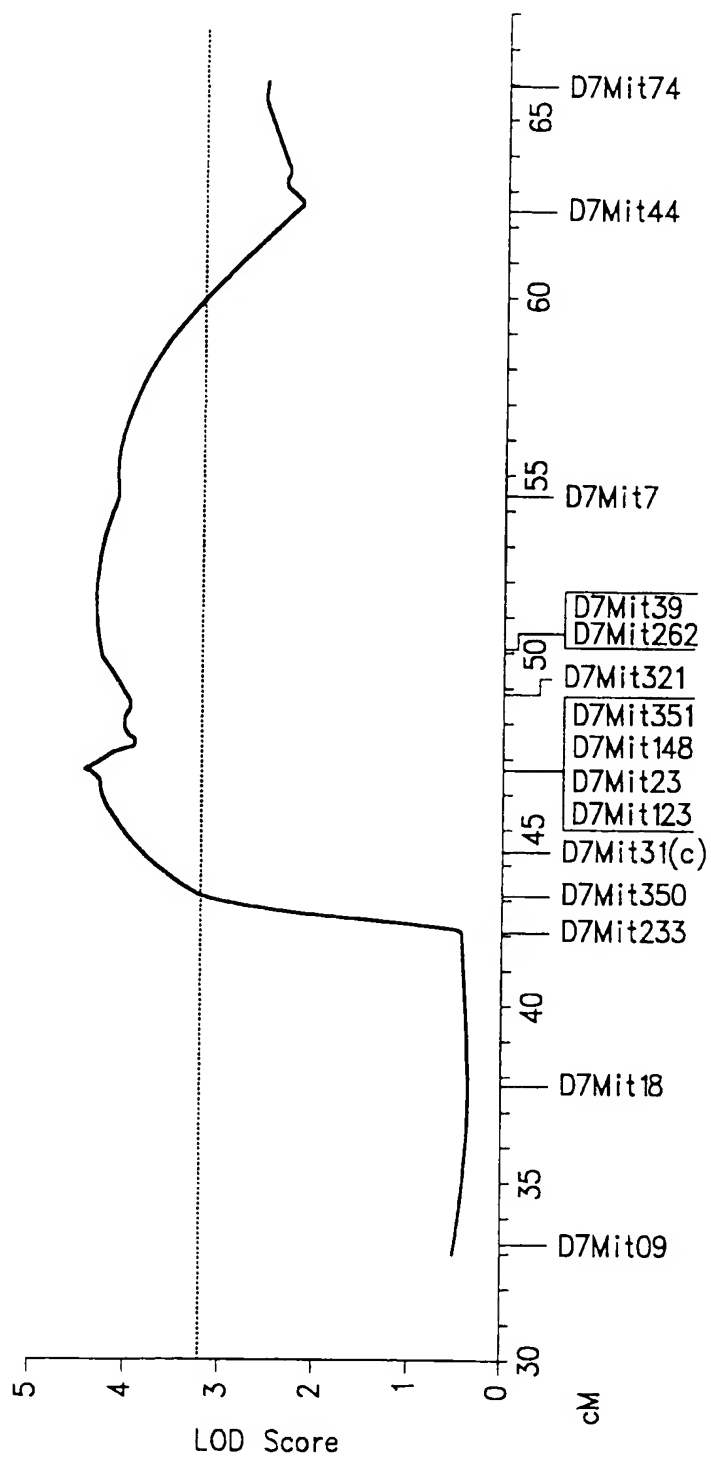
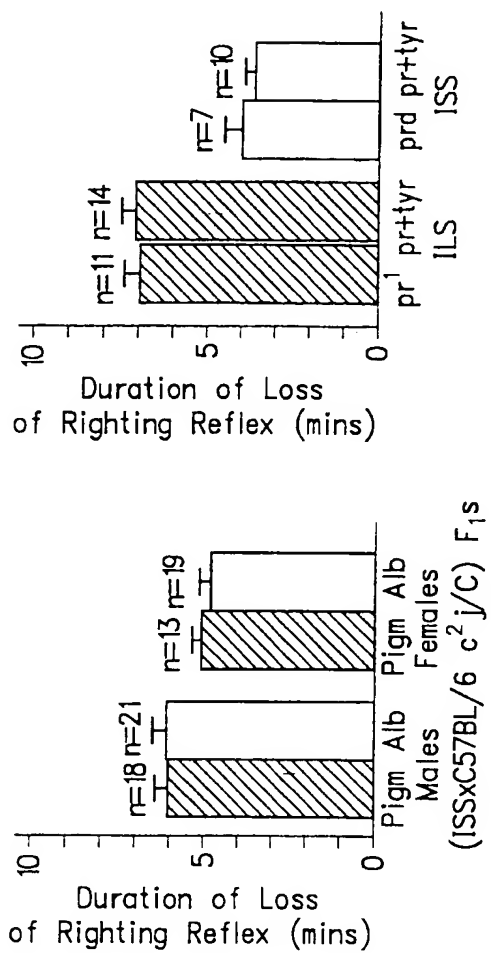


FIG. 6



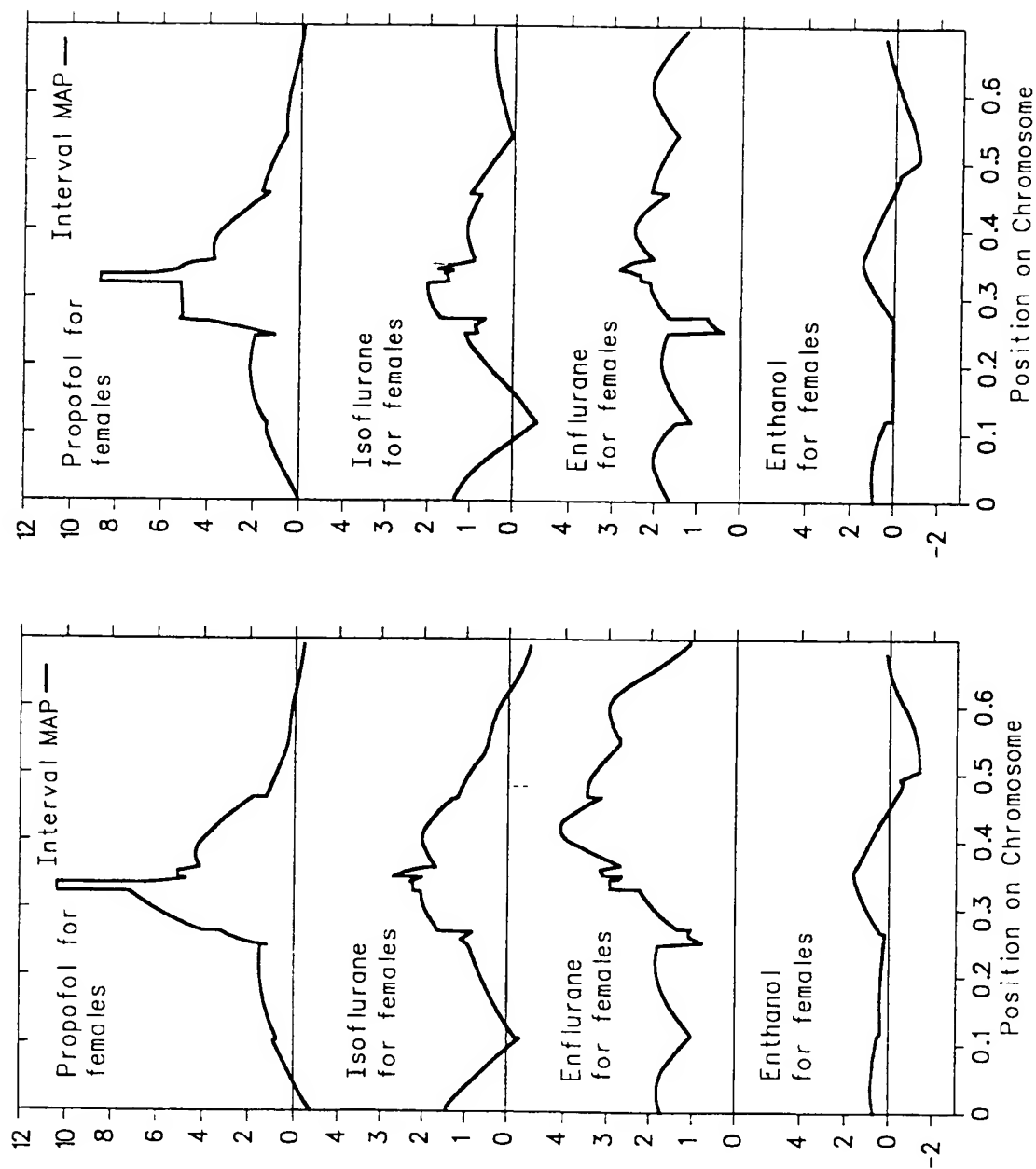


FIG. 8

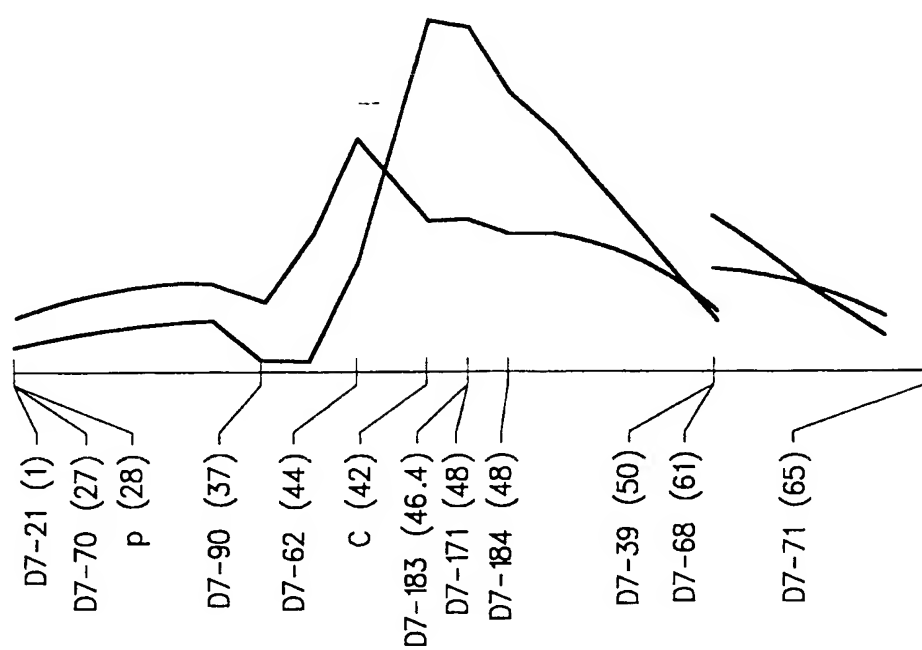


FIG. 9

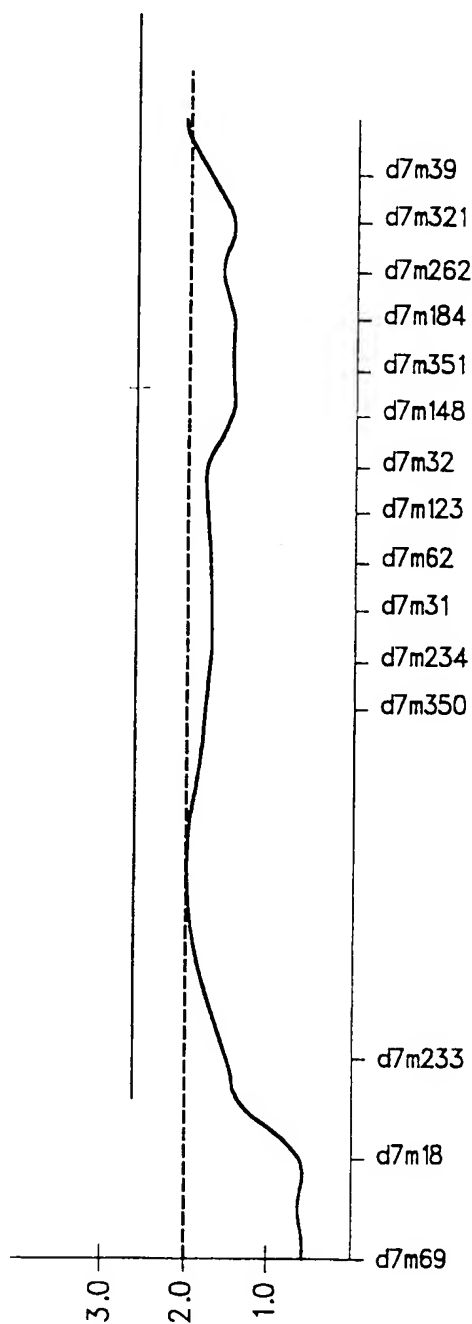


FIG. 10

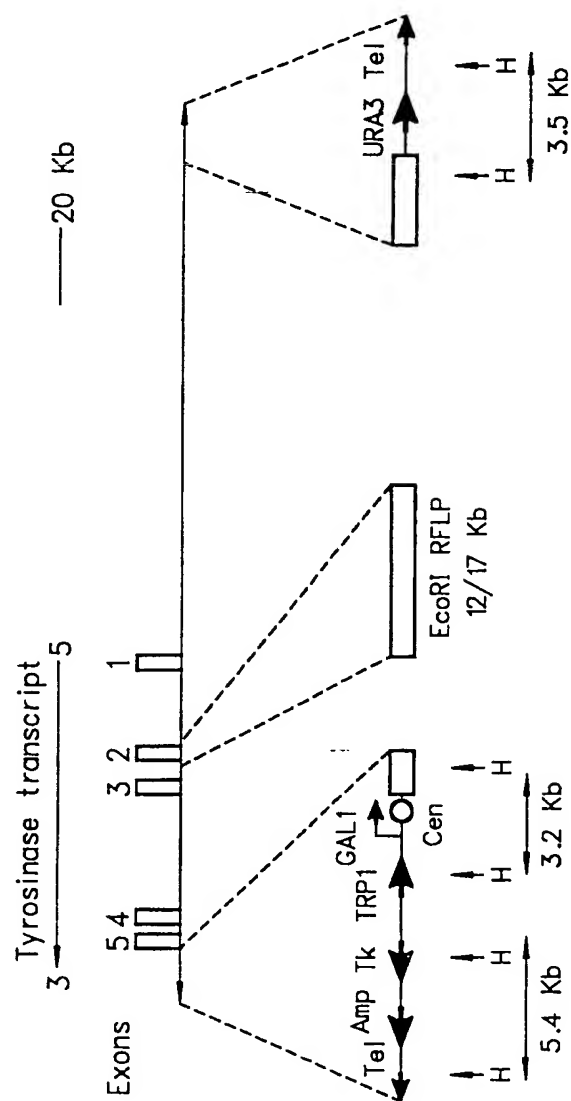


FIG. 11

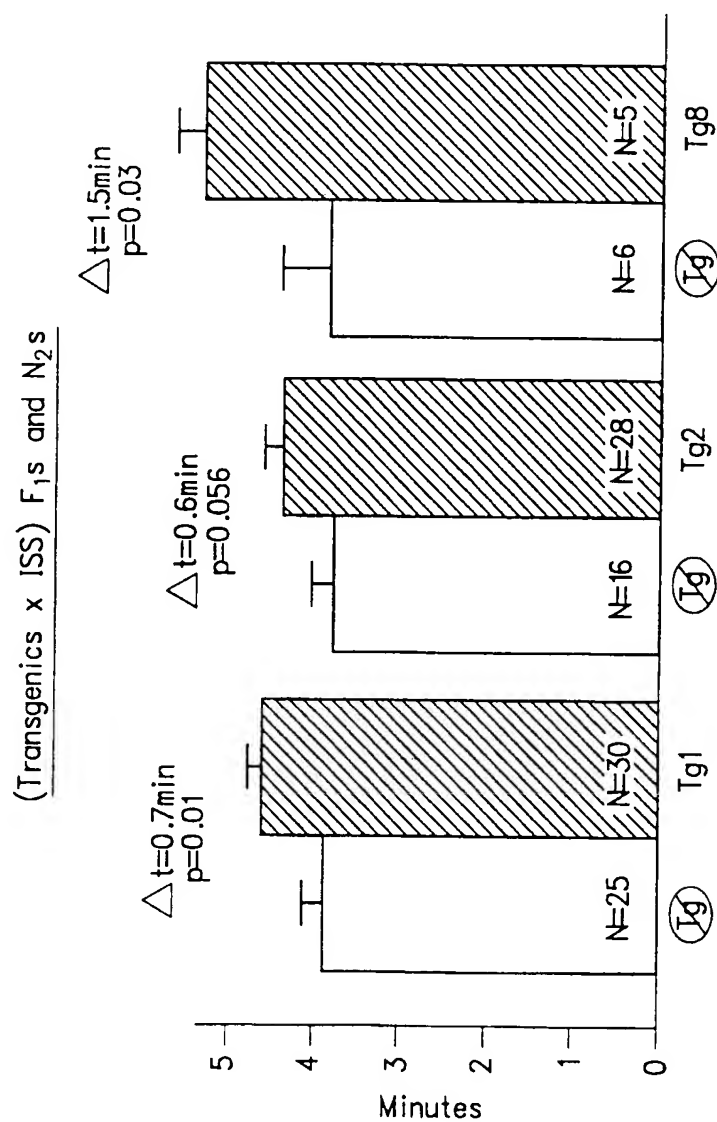


FIG. 12

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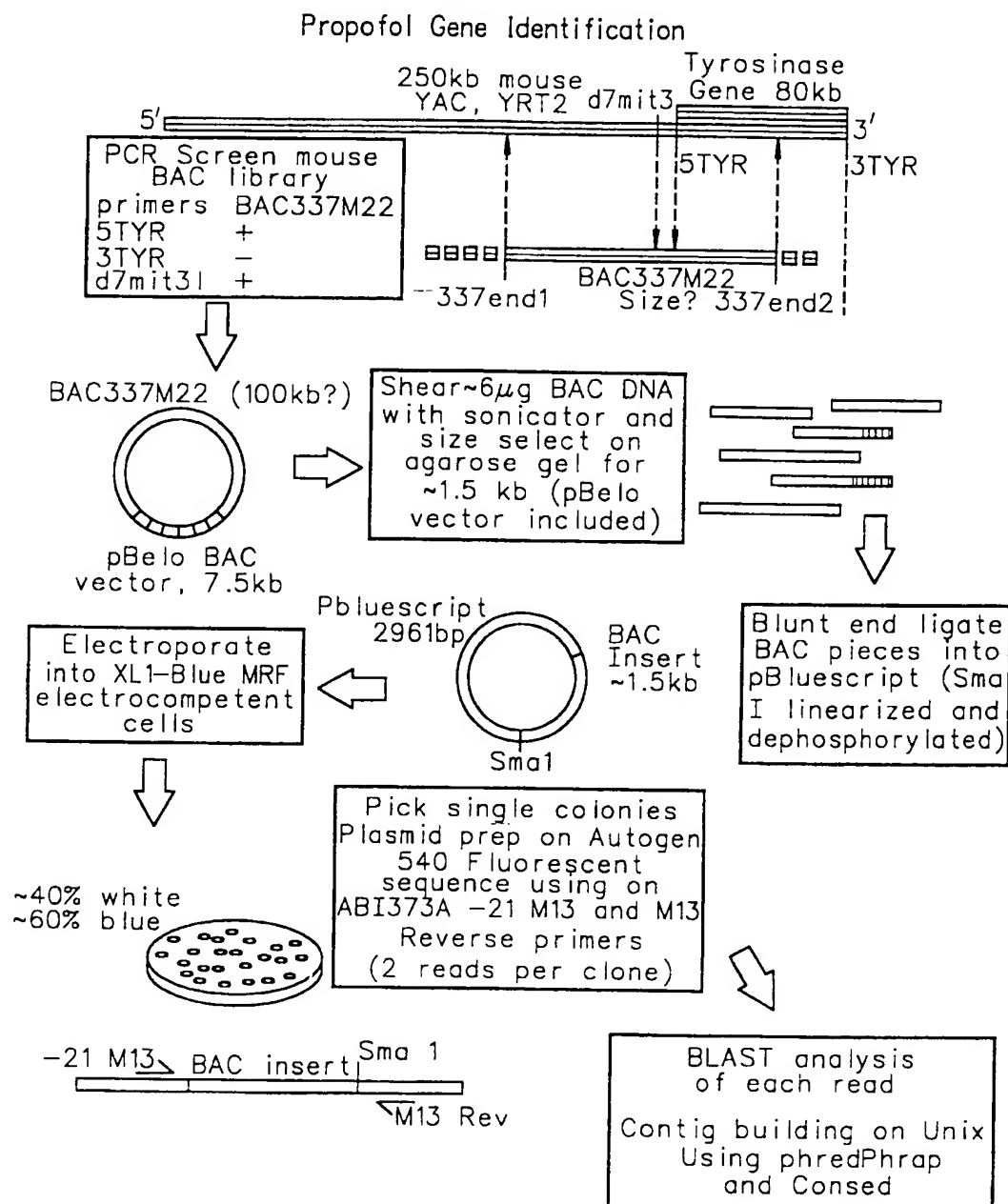


FIG. 13

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mouse mGluR5

ATG GTC CTT CTG TTG ATT CTG TCA GTC CTA CTT CTG AAA GAA GAT GTA
 48
 Met Val Leu Leu Leu Ile Leu Ser Val Leu Leu Leu Lys Glu Asp Val
 1 5 10 15
 CGA GGA AGT GCA CAG TCC AGT GAG AGG AGG GTG GTG GCT CAC ATG CCA
 96
 Arg Gly Ser Ala Gln Ser Ser Glu Arg Arg Val Val Ala His Met Pro
 20 25 30
 GGT GAC ATT ATT ATT GGA GCT CTC TTC TCG GTC CAC CAC CAA CCA ACT
 144
 Gly Asp Ile Ile Ile Gly Ala Leu Phe Ser Val His His Gln Pro Thr
 35 40 45
 GTG GAC AAA GTT CAT GAG AGC AAG TGT GGG GCA GTC CGT GAG CAG TAT
 192
 Val Asp Lys Val His Glu Arg Lys Cys Gly Ala Val Arg Glu Gln Tyr
 50 55 60
 GGC ATT CAG AGA GTG GAA GCC ATG CTG CAT ACC TTG GAA AGG ATC AAT
 240
 Gly Ile Gln Arg Val Glu Ala Met Leu His Thr Leu Glu Arg Ile Asn
 65 70 75 80
 TCA GAT CCC ACA CTC TTG CCC AAC ATC ACA CTA GGC TGT GAG ATA AGA
 288
 Ser Asp Pro Thr Leu Leu Pro Asn Ile Thr Leu Gly Cys Glu Ile Arg
 85 90 95
 GAT TCC TGC TGG CAT TCT GCT GTG GCC CTA GAG CAA AGC ATT GAG TTT
 336
 Asp Ser Cys Trp His Ser Ala Val Ala Leu Glu Gln Ser Ile Glu Phe
 100 105 110
 ATA AGG GAT TCC CTC ATC TCT TCG GAA GAG GAA GAA GGC TTG GTA CGC
 384
 Ile Arg Asp Ser Leu Ile Ser Ser Glu Glu Glu Glu Gly Leu Val Arg
 115 120 125
 TGT GTA GAT GGC TCT TCT TCC TTC CGC TCC AAG AAA CCC ATA GTG GGA
 432
 Cys Val Asp Gly Ser Ser Ser Phe Arg Ser Lys Lys Pro Ile Val Gly
 130 135 140
 GTC ATT GGG CCT GGC TCG AGT TCT GTG GCC ATT CAA GTT CAG AAC TTG
 480
 Val Ile Gly Pro Gly Ser Ser Ser Val Ala Ile Gln Val Gln Asn Leu
 145 150 155 160
 CTC CAG CTT TTC AAC ATA CCT CAG ATT GCT TAC TCT GCA ACT AGC ATG
 528
 Leu Gln Leu Phe Asn Ile Pro Gln Ile Ala Tyr Ser Ala Thr Ser Met
 165 170 175

FIGURE 14(A)

14 / 30

GAT TTG AGT GAC AAG ACT CTA TTC AAG TAC TTC ATG AGG GTT GTA CCT
 576
 Asp Leu Ser Asp Lys Thr Leu Phe Lys Tyr Phe Met Arg Val Val Pro
 180 185 190

TCG GAT GCC CAG CAA GCC CGA GCC ATG GTA GAC ATA GTG AAG AGA TAC
 624
 Ser Asp Ala Gln Gln Ala Arg Ala Met Val Asp Ile Val Lys Arg Tyr
 195 200 205

AAC TGG ACT TAT GTC TCA GCT GTG CAC ACA GAA GGC AAC TAT GCA GAA
 672
 Asn Trp Thr Tyr Val Ser Ala Val His Thr Glu Gly Asn Tyr Gly Glu
 210 215 220

AGT GGG ATG GAG GCT TTC AAA GAT ATG TCA TCG AAG GAA GGG ATT TGC
 720
 Ser Gly Met Glu Ala Phe Lys Asp Met Ser Ala Lys Glu Gly Ile Cys
 225 230 235 240

ATC GCC CAC TCT TAC AAA ATC TAC AGC AAT TCT GGG GAA CAG AGC TTT
 768
 Ile Ala His Ser Tyr Lys Ile Tyr Ser Asn Ala Gly Glu Gln Ser Phe
 245 250 255

GAC AAG CTG TTG AAA AAG CTC AGA AGT CAT TTA CCT AAA GCC CGG GTG
 816
 Asp Lys Leu Leu Lys Lys Leu Arg Ser His Leu Pro Lys Ala Arg Val
 260 265 270

GTA GCC TGC TTC TGT GAA GGC ATG ACA GTT CGA GGT CTG CTC ATG GCC
 864
 Val Ala Cys Phe Cys Glu Gly Met Thr Val Arg Gly Leu Leu Met Ala
 275 280 285

ATG AGA CGC TTG GGT CTA GCA GGG GAA TTT CTA CTT CTG GGC AGT GAT
 912
 Met Arg Arg Leu Gly Leu Ala Gly Glu Phe Leu Leu Leu Gly Ser Asp
 290 295 300

GGC TGG GCT GAC AGG TAT GAC GTG ACA GAT GGG TAT CAG CGA GAA GCT
 960
 Gly Trp Ala Asp Arg Tyr Asp Val Thr Asp Gly Tyr Gln Arg Glu Ala
 305 310 315 320

GTC GGT GGG ATT ACA ATC AAG CTC CAG TCT TCT GAT GTC AAG TGG TTT
 1008
 Val Gly Gly Ile Thr Ile Lys Leu Gln Ser Pro Asp Val Lys Trp Phe
 325 330 335

GAT GAT TAT TAT CTG AAG CTC CGG CCA GAA ACA AAC CTC AGA AAC CCT
 1056
 Asp Asp Tyr Tyr Leu Lys Leu Arg Pro Glu Thr Asn Leu Arg Asn Pro
 340 345 350

FIGURE 14(B)

15 / 30

TGG TTT CAA GAA TTT TGG CAG CAT CGT TTT CAG TGC CGG CTA GAA GGG
 1104
 Trp Phe Gln Glu Phe Trp Gln His Arg Phe Gln Cys Arg Leu Glu Gly
 355 360 365

TTT GCA CAG GAG AAC AGC AAG TAC AAC AAG ACT TGC AAC AGT TCT CTA
 1152
 Phe Ala Gln Glu Asn Ser Lys Tyr Asn Lys Thr Cys Asn Ser Ser Leu
 370 375 380

ACT CTG AGA ACG CAT CAT GTT CAA GAT TCC AAA ATG GGA TTT GTG ATC
 1200
 Thr Leu Arg Thr His His Val Gln Asp Ser Lys Met Gly Phe Val Ile
 385 390 395 400

AAT GCA ATC TAT TCT ATG GCT TAT GGG CTC CAC AAC ATG CAG ATG TCC
 1248
 Asn Ala Ile Tyr Ser Met Ala Tyr Gly Leu His Asn Met Gln Met Ser
 405 410 415

CTG TGT CCA GGC TAT GCA GGC CTC TGT GAT GCA ATG AAG CCA ATT GAT
 1296
 Leu Cys Pro Gly Tyr Ala Gly Leu Cys Asp Ala Met Lys Pro Ile Asp
 420 425 430

GGG CGG AAA CTT TTG GAC TCC CTG ATG AAA ACC AAC TTT ACT GGA GTT
 1344
 Gly Arg Lys Leu Leu Asp Ser Leu Met Lys Thr Asn Phe Thr Gly Val
 435 440 445

TCC GGA GAT ATG ATT CTA TTT GAT GAA AAT GGA GAC TCT CCA GGA AGG
 1392
 Ser Gly Asp Met Ile Leu Phe Asp Glu Asn Gly Asp Ser Pro Gly Arg
 450 455 460

TAT GAA ATA ATG AAT TTC AAG GAA ATG GGA AAA GAT TAT TTT GAT TAC
 1440
 Tyr Glu Ile Met Asn Phe Lys Glu Met Gly Lys Asp Tyr Phe Asp Tyr
 465 470 475 480

ATC AAT GTT GGA AGT TGG GAC AAT GGG GAA TTA AAA ATG GAT GAT GAT
 1488
 Ile Asn Val Gly Ser Trp Asp Asn Gly Glu Leu Lys Met Asp Asp Asp
 485 490 495

GAA GTG TGG TCC AAG AAA AAT AAC ATC ATC AGA TCT GTG TGC AGT GAA
 1536
 Glu Val Trp Ser Lys Lys Asn Asn Ile Ile Arg Ser Val Cys Ser Glu
 500 505 510

CCG TGT GAG AAG GGA CAG ATA AAG GTG ATC CGG AAG GGA GAA GTC AGC
 1584
 Pro Cys Glu Lys Gly Gln Ile Lys Val Ile Arg Lys Gly Glu Val Ser
 515 520 525

FIGURE 14(C)

16 / 30

TGT TGT TGG ACC TGC ACA CCT TGT AAG GAG AAT GAG TAT GTT TTT GAT
 1632
 Cys Cys Trp Thr Cys Thr Pro Cys Lys Glu Asn Glu Tyr Val Phe Asp
 530 535 540

GAG TAC ACC TGC AAG GCG TGC CAA CTG GGG TCC TGG CCC ACT GAC GAC
 1680
 Glu Tyr Thr Cys Lys Ala Cys Gln Leu Gly Ser Trp Pro Thr Asp Asp
 545 550 555 560

TTG ACA GGT TGT GAT TTG ATC CCA GTC CAG TAT CTT CGA TGG GGT GAC
 1728
 Leu Thr Gly Cys Asp Leu Ile Pro Val Gln Tyr Leu Arg Trp Gly Asp
 565 570 575

CCT GAG CCC ATT GCA GCT GTG GTG TTT GCC TGC CTC GGT CTG CTA GCC
 1776
 Pro Glu Pro Ile Ala Ala Val Val Phe Ala Cys Leu Gly Leu Leu Ala
 580 585 590

ACC CTC TTC GTT ACT GTA ATC TTC ATC ATT TAT CGG GAC ACT CCA GTG
 1824
 Thr Leu Phe Val Thr Val Ile Phe Ile Ile Tyr Arg Asp Thr Pro Val
 595 600 605

GTC AAG TCT TCC AGC AGG GAA CTC TGC TAC ATT ATC CTT GCT GGC ATC
 1872
 Val Lys Ser Ser Ser Arg Glu Leu Cys Tyr Ile Ile Leu Ala Gly Ile
 610 615 620

TGC CTG GGT TAC TTG TGT ACC TTC TGC CTC ATT GCA AAG CCC AAA CAG
 1920
 Cys Leu Gly Tyr Leu Cys Thr Phe Cys Leu Ile Ala Lys Pro Lys Gln
 625 630 635 640

ATT TAC TGC TAC CTT CAG AGA ATT GGC ATC GGT CTC TCT CCA GCC ATG
 1968
 Ile Tyr Cys Tyr Leu Gln Arg Ile Gly Ile Gly Leu Ser Pro Ala Met
 645 650 655

AGC TAC TCA GCC CTT GTA ACG AAG ACC AAC CGT ATT GCA AGG ATT CTA
 2016
 Ser Tyr Ser Ala Leu Val Thr Lys Thr Asn Arg Ile Ala Arg Ile Leu
 660 665 670

GCT GGC AGC AAG AAG AAG ATC TGT ACC AAA AAG CCC AGA TTC ATG AGC
 2064
 Ala Gly Ser Lys Lys Lys Ile Cys Thr Lys Lys Pro Arg Phe Met Ser
 675 680 685

GCC TGT GCT CAG TTA GTG ATC GCT TTC ATT CTC ATC TGT ATA CAG TTG
 2112
 Ala Cys Ala Gln Leu Val Ile Ala Phe Ile Leu Ile Cys Ile Gln Leu
 690 695 700

FIGURE 14(D)

17 / 30

GGT ATT ATT GTG GCC CTC TTT ATC ATG GAG CCT CCG GAT ATA ATG CAT
 2160
 Gly Ile Ile Val Ala Leu Phe Ile Met Glu Pro Pro Asp Ile Met His
 705 710 715 720

GAC TAT CCA AGC ATC CGA GAA GTC TAC TTG ATT TGT AAC ACC ACC AAC
 2208
 Asp Tyr Pro Ser Ile Arg Glu Val Tyr Leu Ile Cys Asn Thr Thr Asn
 725 730 735

CTA GGG GTT GTC ACT CCT CTT GGA TAC AAT GGA TTA TTG ATT TTG AGT
 2256
 Leu Gly Val Val Thr Pro Leu Gly Tyr Asn Gly Leu Leu Ile Leu Ser
 740 745 750

TGC ACA TTC TAT GCG TTC AAG ACC AGA AAT GTT CCA GCC AAC TTT AAC
 2304
 Cys Thr Phe Tyr Ala Phe Lys Thr Arg Asn Val Pro Ala Asn Phe Asn
 755 760 765

GAG GCC AAA TAT ATT GCT TTC ACC ATG TAC ACA ACC TGC ATC ATA TGG
 2352
 Glu Ala Lys Tyr Ile Ala Phe Thr Met Tyr Thr Thr Cys Ile Ile Trp
 770 775 780

CTG GCC TTT GTG CCT ATC TAC TTT GGC AGC AAC TAC AAA ATC ATC ACC
 2400
 Leu Ala Phe Val Pro Ile Tyr Phe Gly Ser Asn Tyr Lys Ile Ile Thr
 785 790 795 800

ATG TGT TTC TCA GTC AGC CTC AGT GCC ACA GTG GCC CTG GGT TGC ATG
 2448
 Met Cys Phe Ser Val Ser Leu Ser Ala Thr Val Ala Leu Gly Cys Met
 805 810 815

TTT GTG CCG AAG GTG TAC ATC ATC CTA GCC AAA CCG GAG AGA AAT GTG
 2496
 Phe Val Pro Lys Val Tyr Ile Ile Leu Ala Lys Pro Glu Arg Asn Val
 820 825 830

CGC AGC GCC TTC ACA ACC TCT ACA GTG GTG CGC ATG CAC GTA GGA GAT
 2544
 Arg Ser Ala Phe Thr Thr Ser Thr Val Val Arg Met His Val Gly Asp
 835 840 845

GGC AAG TCA TCA TCC GCT GCC AGC AGA TCC AGC AGC CTA GTC AAC CTG
 2592
 Gly Lys Ser Ser Ser Ala Ala Ser Arg Ser Ser Ser Leu Val Asn Leu
 850 855 860

TGG AAG AGG AGG GGC TCG TCT GGG GAA ACC CTA AGG TAC AAA GAC AGC
 2640
 Trp Lys Arg Arg Gly Ser Ser Gly Glu Thr Leu Arg Tyr Lys Asp Arg
 865 870 875 880

FIGURE 14(E)

18 / 30

AGA CTG GCC CAG CAC AAG TCG GAA ATA GAG TGT TTC ACC CCC AAA GGG
 2688
 Arg Leu Ala Gln His Lys Ser Glu Ile Glu Cys Phe Thr Pro Lys Gly
 885 890 895

AGT ATG GGG AAT GGT GGG AGA GCA ACA ATG AGC AGC TCC AAT GSA AAA
 2736
 Ser Met Gly Asn Gly Gly Arg Ala Thr Met Ser Ser Ser Asn Gly Lys
 900 905 910

TCC GTG ACT TGG GCC CAG AAT GAG AAG AGC ACC CGG GGG CAG CAC CTG
 2784
 Ser Val Thr Trp Ala Gln Asn Glu Lys Ser Thr Arg Gly Gln His Leu
 915 920 925

TGG CAG CGA CTG TCT GTC CAT ATC AAC AAG AAG GAG AAC CCC AAC CAG
 2832
 Trp Gln Arg Leu Ser Val His Ile Asn Lys Lys Glu Asn Pro Asn Gln
 930 935 940

ACA GCA GTC ATC AAA CCC TTC CCC AAG AGC ACA GAG AGC CGC GGG CAG
 2880
 Thr Ala Val Ile Lys Pro Phe Pro Lys Ser Thr Glu Ser Arg Gly Gln
 945 950 955 960

GGT GCA GGG GCA GGT GGT GGC TCT GGC CCC GGT GCA GCT GGT GCT GGT
 2928
 Gly Ala Gly Ala Gly Gly Gly Ser Gly Pro Gly Ala Ala Gly Ala Gly
 965 970 975

AGC GCA GGA TGC ACA GCG ACA GGC GGC CCA GAG CCA CCA GAC CCC GGC
 2976
 Ser Ala Gly Cys Thr Ala Thr Gly Gly Pro Glu Pro Pro Asp Ala Gly
 980 985 990

CCC AAG GCG CTT TAT GAT GTC GCA GAG GCA GAG GAG CGC TTC CCA GCG
 3024
 Pro Lys Ala Leu Tyr Asp Val Ala Glu Ala Glu Glu Arg Phe Pro Ala
 995 1000 1005

GCT GCC AGG CCG CGC TCG CCA TCG CCC ATC AGT ACG CTG AGC CAC CTG
 3072
 Ala Ala Arg Pro Arg Ser Pro Ser Pro Ile Ser Thr Leu Ser His Leu
 1010 1015 1020

GCA GGC TCG GCG GGC CGC ACA GAC GAC GAC GCG CCG TCG CTG CAC TCG
 3120
 Ala Gly Ser Ala Gly Arg Thr Asp Asp Asp Ala Pro Ser Leu His Ser
 1025 1030 1035 1040

GAG ACC GCT GCA CGC AGC AGC TCA TCC CAG GGC TCG CTG ATG GAG CAG
 3168
 Glu Thr Ala Ala Arg Ser Ser Ser Ser Gln Gly Ser Leu Met Glu Gln
 1045 1050 1055

FIGURE 14(F)

19 / 30

ATT AGC AGC GTG GTG ACG CGC TTC ACC GCC AAC ATC ACC GAG CTC AAC
 3216
 Ile Ser Ser Val Val Thr Arg Phe Thr Ala Asn Ile Thr Glu Leu Asn
 1060 1065 1070

TCC ATG ATG TTG TCC ACC GCG GCT GCG CCG GGG CCC CCT GGT ACC CCT
 3264
 Ser Met Met Leu Ser Thr Ala Ala Ala Pro Gly Pro Pro Gly Thr Pro
 1075 1080 1085

ATC TGC TCT TCC TAC CTG ATC CCC AAA GAG ATC CAG CTG CCC ACG ACC
 3312
 Ile Cys Ser Ser Tyr Leu Ile Pro Lys Glu Ile Gln Leu Pro Thr Thr
 1090 1095 1100

ATG ACG ACC TTC GCA GAG ATC CAG CCT CTG CCG GCC ATC GAG GTG ACC
 3360
 Met Thr Thr Phe Ala Glu Ile Gln Pro Leu Pro Ala Ile Glu Val Thr
 1105 1110 1115 1120

GGA GGA GCT CAG CCG GCG ACA GGG CCA TCA CCT GCC CAA GAG ACG CCC
 3408
 Gly Gly Ala Gln Pro Ala Thr Gly Pro Ser Pro Ala Gln Glu Thr Pro
 1125 1130 1135

GCA GGA GCT GAA GCC GCC CCA GGA AAA CCG GAT CTG GAG GAG CTG GTG
 3456
 Ala Gly Ala Glu Ala Ala Pro Gly Lys Pro Asp Leu Glu Glu Leu Val
 1140 1145 1150

GCC CTC ACT CCA CCA TCG CCC TTC AGG GAC TCG GTG GAC TCG GGG AGC
 3504
 Ala Leu Thr Pro Pro Ser Pro Phe Arg Asp Ser Val Asp Ser Gly Ser
 1155 1160 1165

ACC ACC CCA AAC TCT CCA GTC TCC GAA TCG GCC CTC TGC ATC CCA TCC
 3552
 Thr Thr Pro Asn Ser Pro Val Ser Glu Ser Ala Leu Cys Ile Pro Ser
 1170 1175 1180

TCT CCC AAA TAT GAC ACT CTC ATC ATC AGA GAT TAC ACG
 3591
 Ser Pro Lys Tyr Asp Thr Leu Ile Ile Arg Asp Tyr Thr
 1185 1190 1195

FIGURE 14(G)

20 / 30

Portion of mouse mGluR5 from Genbank database

AG GTA CAA AGA CAG GAG ACT GGC CCA GCA CAA GTC GGA AAT AGA GTG
47
Val Gln Arg Gln Glu Thr Gly Pro Ala Gln Val Gly Asn Arg Val
1 5 10 15
TTT CAC CCC CAA AGG GAG TAT GGG GAA TGG TGG GAG AGC AAC AAT GAG
95
Phe His Pro Gln Arg Glu Tyr Gly Glu Trp Trp Glu Ser Asn Asn Glu
20 25 30
CAG GT
100
Gln

FIGURE 15

21 / 30

rat mGluR5

TCAGACATCT CTTGAGAAAC TCAGCCTAGT TTATCCAGCT GTTTGTCCA CATATGTTCA
60
TAAAATCTAG TTATGAATAT TCTGATCGTA TGGATGCACC TAACTCGACA GTTCTGGGG
120
ACTTTGCTAT CTTT TAGAGA ATATATCTAA ATTGCTGACT AATTCCTCAA TCGGCCACTC
180
AACGCAGGGC ATCATTGGC TGTACCTATT GGAGCTCTGA GTTCCCTCCA TCATCATATC
240
TTTATTAGCT TGAATTCCTT TCCTAAA ATG GTC CTT CTG TTG ATC CTG TCA GTC
294

Met Val Leu Leu Leu Ile Leu Ser Val
1 5

CTA CTT CTG AAA GAA GAT GTA CGA GGG AGT GCA CAG TCC AST GAG AGG
342
Leu Leu Leu Lys Glu Asp Val Arg Gly Ser Ala Gln Ser Ser Glu Arg
10 15 20 25

AGG GTG GTG GCT CAC ATG CCA GGT GAC ATC ATT ATT GGA GCT CTC TTC
390
Arg Val Val Ala His Met Pro Gly Asp Ile Ile Ile Gly Ala Leu Phe
30 35 40

TCT GTC CAC CAC CAA CCA ACT GTG GAC AAA GTT CAT GAG AGG AAG TGT
438
Ser Val His His Gln Pro Thr Val Asp Lys Val His Glu Arg Lys Cys
45 50 55

GGT GCA GTT CGT GAG CAA TAT GGG ATT CAG AGA GTG GAG GCC ATG CTG
486
Gly Ala Val Arg Glu Gln Tyr Gly Ile Gln Arg Val Glu Ala Met Leu
60 65 70

CAT ACC TTG GAA AGG ATC AAC TCA GAT CCC ACA CTC TTG CCC AAC ATC
534
His Thr Leu Glu Arg Ile Asn Ser Asp Pro Thr Leu Leu Pro Asn Ile
75 80 85

ACA CTT GGC TGT GAG ATC AGA GAT TCC TGC TGG CAT TCC GCT GTG GCC
582
Thr Leu Gly Cys Glu Ile Arg Asp Ser Cys Trp His Ser Ala Val Ala
90 95 100 105

CTA GAA CAA AGC ATT GAG TTT ATA AGG GAT TCC CTC ATC TCT TCA GAA
630
Leu Glu Gln Ser Ile Glu Phe Ile Arg Asp Ser Leu Ile Ser Ser Glu
110 115 120

GAG GAG GAA GGT TTG GTA CGC TGT GTA GAT GGA TCT TCT TCC TTC CGC
678
Glu Glu Glu Gly Leu Val Arg Cys Val Asp Gly Ser Ser Ser Phe Arg
125 130 135

FIGURE 16(A)

22 / 30

TCC AAG AAA CCC ATA GTG GGA GTC ATT GGG CCT GGC TCG AGT TCT GTG
 726
 Ser Lys Lys Pro Ile Val Gly Val Ile Gly Pro Gly Ser Ser Ser Val
 140 145 150

GCC ATT CAA GTC CAG AAC TTA CTC CAG CTT TTC AAC ATA CCA CAG ATT
 774
 Ala Ile Gln Val Gln Asn Leu Leu Gln Leu Phe Asn Ile Pro Gln Ile
 155 160 165

GCT TAC TCT GCA ACC AGC ATG GAT TTG AGT GAC AAG ACT CTA TTC AAG
 822
 Ala Tyr Ser Ala Thr Ser Met Asp Leu Ser Asp Lys Thr Leu Phe Lys
 170 175 180 185

TAC TTC ATG AGG GTT GTA CCT TCC GAT GCC CAG CAA GCT CGA GCC ATG
 870
 Tyr Phe Met Arg Val Val Pro Ser Asp Ala Gln Gln Ala Arg Ala Met
 190 195 200

GTA GAC ATA GTG AAG AGA TAC AAC TGG ACT TAT GTC TCA GCT GTG CAC
 918
 Val Asp Ile Val Lys Arg Tyr Asn Trp Thr Tyr Val Ser Ala Val His
 205 210 215

ACA GAA GGC AAC TAT GGA GAA AGT GGG ATG GAG GCT TTC AAA GAT ATG
 966
 Thr Glu Gly Asn Tyr Gly Glu Ser Gly Met Glu Ala Phe Lys Asp Met
 220 225 230

TCA GCG AAG GAA GGG ATT TGC ATC GCC CAC TCT TAC AAA ATT TAC AGC
 1014
 Ser Ala Lys Glu Gly Ile Cys Ile Ala His Ser Tyr Lys Ile Tyr Ser
 235 240 245

AAT GCT GGG GAA CAG AGC TTT GAC AAG CTG TTG AAA AAA CTC AGA AGT
 1062
 Asn Ala Gly Glu Gln Ser Phe Asp Lys Leu Leu Lys Lys Leu Arg Ser
 250 255 260 265

CAT TTA CCT AAA GCC CGG GTG GTA GCT TGC TTC TGT GAA GGC ATG ACA
 1110
 His Leu Pro Lys Ala Arg Val Val Ala Cys Phe Cys Glu Gly Met Thr
 270 275 280

GTT CGA GGT CTG CTC ATG GCC ATG AGA CGC TTA GGT CTA GCA GGG GAG
 1158
 Val Arg Gly Leu Leu Met Ala Met Arg Arg Leu Gly Leu Ala Gly Glu
 285 290 295

TTT CTA CTT CTG GGC AGT GAT GGC TGG GCC GAC AGG TAT GAC GTG ACA
 1206
 Phe Leu Leu Leu Gly Ser Asp Gly Trp Ala Asp Arg Tyr Asp Val Thr
 300 305 310

FIGURE 16(B)

23 / 30

GAT GGA TAT CAG CGA GAA GCT GTC GGT GGA ATT ACA ATC AAG CTT CAG
 1254
 Asp Gly Tyr Gln Arg Glu Ala Val Gly Gly Ile Thr Ile Lys Leu Gln
 315 320 325

TCT CCT GAT GTC AAG TGG TTT GAT GAT TAT TAT CTG AAA CTC CGG CCA
 1302
 Ser Pro Asp Val Lys Trp Phe Asp Asp Tyr Tyr Leu Lys Leu Arg Pro
 330 335 340 345

GAA ACA AAC CTC AGA AAC CCT TGG TTT CAA GAA TTT TGG CAG CAT CGT
 1350
 Glu Thr Asn Leu Arg Asn Pro Trp Phe Gln Glu Phe Trp Gln His Arg
 350 355 360

TTT CAG TGC CGG CTG GAA GGG TTT GCA CAG GAG AAC AGC AAG TAC AAC
 1398
 Phe Gln Cys Arg Leu Glu Gly Phe Ala Gln Glu Asn Ser Lys Tyr Asn
 365 370 375

AAG ACT TGC AAC AGT TCT CTG ACT CTG AGA ACA CAT CAT GTT CAA GAT
 1446
 Lys Thr Cys Asn Ser Ser Leu Thr Leu Arg Thr His His Val Gln Asp
 380 385 390

TCC AAA ATG GGA TTT GTG ATA AAT GCA ATC TAT TCT ATG GCT TAT GGA
 1494
 Ser Lys Met Gly Phe Val Ile Asn Ala Ile Tyr Ser Met Ala Tyr Gly
 395 400 405

CTC CAC AAT ATG CAG ATG TCC CTG TGT CCA GGC TAT GCA GGC CTC TGT
 1542
 Leu His Asn Met Gln Met Ser Leu Cys Pro Gly Tyr Ala Gly Leu Cys
 410 415 420 425

GAT GCG ATG AAG CCA ATT GAT GGA CGG AAA CTT TTG GAT TCC CTG ATG
 1590
 Asp Ala Met Lys Pro Ile Asp Gly Arg Lys Leu Leu Asp Ser Leu Met
 430 435 440

AAA ACC AAT TTT ACT GGA GTT TTT GGA GAT ATG ATC CTG TTT GAT GAA
 1638
 Lys Thr Asn Phe Thr Gly Val Ser Gly Asp Met Ile Leu Phe Asp Glu
 445 450 455

AAT GGA GAC TCT CCA GGA AGG TAT GAA ATA ATG AAT TTC AAG GAA ATG
 1686
 Asn Gly Asp Ser Pro Gly Arg Tyr Glu Ile Met Asn Phe Lys Glu Met
 460 465 470

GGA AAA GAT TAT TTT GAT TAC ATC AAT GTT GGA AGT TGG GAC AAT GGG
 1734
 Gly Lys Asp Tyr Phe Asp Tyr Ile Asn Val Gly Ser Trp Asp Asn Gly
 475 480 485

FIGURE 16(C)

24 / 30

GAA TTA AAA ATG GAT GAT GAC GAA GTG TGG TCC AAG AAA AAT AAC ATC
 1782
 Glu Leu Lys Met Asp Asp Asp Glu Val Trp Ser Lys Lys Asn Asn Ile
 490 495 500 505

ATC AGA TCT GTG TGC AGT GAA CCA TGT GAG AAA GGC CAA ATA AAG GTG
 1830
 Ile Arg Ser Val Cys Ser Glu Pro Cys Glu Lys Gly Gln Ile Lys Val
 510 515 520

ATC CGG AAG GGA GAA GTC AGC TGT TGT TGG ACC TGC ACA CCT TGT AAA
 1878
 Ile Arg Lys Gly Glu Val Ser Cys Cys Trp Thr Cys Thr Pro Cys Lys
 525 530 535

GAG AAT GAG TAT GTG TTT GAT GAG TAC ACC TGC AAG GCA TGC CAA CTG
 1926
 Glu Asn Glu Tyr Val Phe Asp Glu Tyr Thr Cys Lys Ala Cys Gln Leu
 540 545 550

GGC TCC TGG CCC ACT GAC GAC CTG ACA GGC TGT GAC TTG ATC CCA GTC
 1974
 Gly Ser Trp Pro Thr Asp Asp Leu Thr Gly Cys Asp Leu Ile Pro Val
 555 560 565

CAG TAT CTT CGC TGG GGT GAC CCT GAG CCG ATT GCA GCT GTG GTG TTT
 2022
 Gln Tyr Leu Arg Trp Gly Asp Pro Glu Pro Ile Ala Ala Val Val Phe
 570 575 580 585

GCC TGC CTC GGT CTG CTA GCC ACC TTA TTC GTT ACT GTA ATC TTC ATC
 2070
 Ala Cys Leu Gly Leu Leu Ala Thr Leu Phe Val Thr Val Ile Phe Ile
 590 595 600

ATT TAT CGG GAC ACT CCG GTG GTC AAG TCC TCC AGC AGG GAG CTC TGC
 2118
 Ile Tyr Arg Asp Thr Pro Val Val Lys Ser Ser Ser Arg Glu Leu Cys
 605 610 615

TAC ATT ATC CTT GCT GGC ATC TGC CTG GGA TAC TTA TGT ACC TTT TGC
 2166
 Tyr Ile Ile Leu Ala Gly Ile Cys Leu Gly Tyr Leu Cys Thr Phe Cys
 620 625 630

CTC ATT GCA AAG CCC AAG CAG ATT TAC TGC TAT CTT CAG AGA ATT GGC
 2214
 Leu Ile Ala Lys Pro Lys Gln Ile Tyr Cys Tyr Leu Gln Arg Ile Gly
 635 640 645

ATT GGA CTC TCT CCA GCC ATG AGC TAC TCA GCC CTT GTA ACC AAG ACC
 2262
 Ile Gly Leu Ser Pro Ala Met Ser Tyr Ser Ala Leu Val Thr Lys Thr
 650 655 660 665

FIGURE 16(D)

25 / 30

AAC CGT ATT GCA AGG ATT CTA GCT GGA AGC AAG AAG AAA ATC TGT ACC
 2310
 Asn Arg Ile Ala Arg Ile Leu Ala Gly Ser Lys Lys Lys Ile Cys Thr
 670 675 680

AAG AAG CCC AGA TTC ATG AGC GCC TGT GCT CAG TTA GTG ATC GCT TTC
 2358
 Lys Lys Pro Arg Phe Met Ser Ala Cys Ala Gln Leu Val Ile Ala Phe
 685 690 695

ATT CTC ATC TGT ATT CAG CTG GGC ATT ATC GTG GCC CTC TTT ATC ATG
 2406
 Ile Leu Ile Cys Ile Gln Leu Gly Ile Ile Val Ala Leu Phe Ile Met
 700 705 710

GAG CCT CCG GAT ATA ATG CAT GAC TAT CCA AGC ATC CGA GAA GTC TAC
 2454
 Glu Pro Pro Asp Ile Met His Asp Tyr Pro Ser Ile Arg Glu Val Tyr
 715 720 725

TTG ATT TGT AAC ACC ACC AAC CTA GGG GTT GTT ACT CCT CTT GGA TAC
 2502
 Leu Ile Cys Asn Thr Thr Asn Leu Gly Val Val Thr Pro Leu Gly Tyr
 730 735 740 745

AAT GGA TTA TTG ATT TTG AGT TGC ACG TTC TAT GCG TTT AAG ACC AGA
 2550
 Asn Gly Leu Leu Ile Leu Ser Cys Thr Phe Tyr Ala Phe Lys Thr Arg
 750 755 760

AAT GTT CCA GCC AAC TTT AAT GAG GCC AAA TAT ATT GCT TTC ACC ATG
 2598
 Asn Val Pro Ala Asn Phe Asn Glu Ala Lys Tyr Ile Ala Phe Thr Met
 765 770 775

TAC ACA ACC TGC ATC ATA TGG CTG GCC TTC GTG CCT ATC TAC TTT GGC
 2646
 Tyr Thr Thr Cys Ile Ile Trp Leu Ala Phe Val Pro Ile Tyr Phe Gly
 780 785 790

AGC AAC TAC AAA ATC ATC ACC ATG TGT TTT TCA GTC AGC CTC AGT GCC
 2694
 Ser Asn Tyr Lys Ile Ile Thr Met Cys Phe Ser Val Ser Leu Ser Ala
 795 800 805

ACA GTG GCC CTG GGT TGC ATG TTT GTC CCG AAG GTG TAC ATC ATC CTA
 2742
 Thr Val Ala Leu Gly Cys Met Phe Val Pro Lys Val Tyr Ile Ile Leu
 810 815 820 825

GCC AAA CCG GAG AGA AAT GTG CGC AGC GCC TTC ACA ACC TCT ACA GTG
 2790
 Ala Lys Pro Glu Arg Asn Val Arg Ser Ala Phe Thr Thr Ser Thr Val
 830 835 840

FIGURE 16(E)

GTA CGC ATG CAT GTA GGA GAC GGC AAA TCA TCG TCC GCT GCC AGC AGA
 2838
 Val Arg Met His Val Gly Asp Gly Lys Ser Ser Ser Ala Ala Ser Arg
 845 850 855

TCC AGC AGC CTA GTC AAC CTG TGG AAG AGG AGG GGC TCC TCT GGG GAA
 2886
 Ser Ser Ser Leu Val Asn Leu Trp Lys Arg Arg Gly Ser Ser Gly Glu
 860 865 870

ACC CTA AGC TCC AAC GGA AAA TCT GTG ACT TGG GCC CAG AAT GAG AAG
 2934
 Thr Leu Ser Ser Asn Gly Lys Ser Val Thr Trp Ala Gln Asn Glu Lys
 875 880 885

AGT ACC CGG GGG CAA CAT TTG TGG CAG CGA CTG TCT GTC CAC ATC AAC
 2982
 Ser Thr Arg Gly Gln His Leu Trp Gln Arg Leu Ser Val His Ile Asn
 890 895 900 905

AAG AAG GAG AAC CCC AAC CAA ACG GCG GTC ATC AAA CCC TTT CCC AAG
 3030
 Lys Lys Glu Asn Pro Asn Gln Thr Ala Val Ile Lys Pro Phe Pro Lys
 910 915 920

AGC ACA GAG AAC CGG GGG CCT GGT GCA GCG GCA GGT GGT GGC TCG GGT
 3078
 Ser Thr Glu Asn Arg Gly Pro Gly Ala Ala Ala Gly Gly Gly Ser Gly
 925 930 935

CCC GGT GTA GCT GGT GCT GGT AAC GCA GGA TGC ACA GCA ACA GGT GGC
 3126
 Pro Gly Val Ala Gly Ala Gly Asn Ala Gly Cys Thr Ala Thr Gly Gly
 940 945 950

CCG GAG CCA CCG GAC GCC GGT CCC AAA GCG CTT TAT GAT GTG GCG GAG
 3174
 Pro Glu Pro Pro Asp Ala Gly Pro Lys Ala Leu Tyr Asp Val Ala Glu
 955 960 965

GCA GAG GAG AGC TTC CCC GCG GCT GCC AGG CCG CGC TCG CCA TCG CCC
 3222
 Ala Glu Glu Ser Phe Pro Ala Ala Ala Arg Pro Arg Ser Pro Ser Pro
 970 975 980 985

ATC AGC ACG TTG AGC CAC CTC GCA GGC TCG GCG GGC CGC ACA GAT GAT
 3270
 Ile Ser Thr Leu Ser His Leu Ala Gly Ser Ala Gly Arg Thr Asp Asp
 990 995 1000

GAT GCG CCG TCG CTG CAC TCT GAG ACA GCT GCT CGC AGC AGC TCG TCC
 3318
 Asp Ala Pro Ser Leu His Ser Glu Thr Ala Ala Arg Ser Ser Ser Ser
 1005 1010 1015

FIGURE 16(F)

27 / 30

CAG GGC TCG CTC ATG GAG CAG ATC AGC AGC GTA GTG ACG CGC TTC ACA
 3366
 Gln Gly Ser Leu Met Glu Gln Ile Ser Ser Val Val Thr Arg Phe Thr
 1020 1025 1030

GCC AAC ATC TCC GAG CTC AAC TCC ATG ATG TTG TCC ACC GCG GCC ACG
 3414
 Ala Asn Ile Ser Glu Leu Asn Ser Met Met Leu Ser Thr Ala Ala Thr
 1035 1040 1045

CCG GGG CCC CCT GGT ACT CCA ATC TGC TCC TCC TAC CTG ATC CCC AAA
 3462
 Pro Gly Pro Pro Gly Thr Pro Ile Cys Ser Ser Tyr Leu Ile Pro Lys
 1050 1055 1060 1065

GAG ATT CAG CTG CCC ACG ACC ATG ACG ACC TTC GCA GAG ATC CAG CCG
 3510
 Glu Ile Gln Leu Pro Thr Thr Met Thr Thr Phe Ala Glu Ile Gln Pro
 1070 1075 1080

CTG CCG GCC ATC GAG GTG ACC GGA GGA GCT CAG GGG GCG ACA GGC GTA
 3558
 Leu Pro Ala Ile Glu Val Thr Gly Gly Ala Gln Gly Ala Thr Gly Val
 1085 1090 1095

TCA CCT GCC CAG GAG ACG CCC ACA GGA GCT GAA TCC GCC CCG GGC AAA
 3606
 Ser Pro Ala Gln Glu Thr Pro Thr Gly Ala Glu Ser Ala Pro Gly Lys
 1100 1105 1110

CCA GAT CTG GAG GAG CTT GTG GCC CTC ACT CCA CCA TCG CCC TTC AGG
 3654
 Pro Asp Leu Glu Glu Leu Val Ala Leu Thr Pro Pro Ser Pro Phe Arg
 1115 1120 1125

GAC TCG GTG GAC TCG GGG AGC ACC ACC CCA AAC TCT CCA GTC TCA GAA
 3702
 Asp Ser Val Asp Ser Gly Ser Thr Thr Pro Asn Ser Pro Val Ser Glu
 1130 1135 1140 1145

TCG GCC CTC TGC ATC CCA TCC TCT CCC AAA TAT GAC ACT CTC ATC ATC
 3750
 Ser Ala Leu Cys Ile Pro Ser Ser Pro Lys Tyr Asp Thr Leu Ile Ile
 1150 1155 1160

AGA GAT TAC ACG CAG AGT TCT TCA TCG TTG TGAGCCACTG GAAACTTCCC TGA
 3803
 Arg Asp Tyr Thr Gln Ser Ser Ser Ser Leu
 1165 1170

GATAGGCCTG TGCAGCAGAC CTCGGTGTTC CAGATGGGCG CAGCGACACC CATAGTCACC
 3863
 CCCACAGAGA ACCCGAGGTG AAAAAATGCCA AGGACACACA CGGTGGAGAC ATGAG
 3918

FIGURE 16(G)

human mGluR5

Met Val Leu Leu Leu Ile Leu Ser Val Leu Leu Leu Lys Glu Asp Val
 1 5 10 15
 Arg Gly Ser Ala Gln Ser Ser Glu Arg Arg Val Val Ala His Met Pro
 20 25 30
 Gly Asp Ile Ile Ile Gly Ala Leu Phe Ser Val His His Gln Pro Thr
 35 40 45
 Val Asp Lys Val His Glu Arg Lys Cys Gly Ala Val Arg Glu Gln Tyr
 50 55 60
 Gly Ile Gln Arg Val Glu Ala Met Leu His Thr Leu Glu Arg Ile Asn
 65 70 75 80
 Ser Asp Pro Thr Leu Leu Pro Asn Ile Thr Leu Gly Cys Glu Ile Arg
 85 90 95
 Asp Ser Cys Trp His Ser Ala Val Ala Leu Glu Gln Ser Ile Glu Phe
 100 105 110
 Ile Arg Asp Ser Leu Ile Ser Ser Glu Glu Glu Glu Gly Leu Val Arg
 115 120 125
 Cys Val Asp Gly Ser Ser Ser Ser Phe Arg Ser Lys Lys Pro Ile Val
 130 135 140
 Gly Val Ile Gly Pro Gly Ser Ser Ser Val Ala Ile Gln Val Gln Asn
 145 150 155 160
 Leu Leu Gln Leu Phe Asn Ile Pro Gln Ile Ala Tyr Ser Ala Thr Ser
 165 170 175
 Met Asp Leu Ser Asp Lys Thr Leu Phe Lys Tyr Phe Met Arg Val Val
 180 185 190
 Pro Ser Asp Ala Gln Gln Ala Arg Ala Met Val Asp Ile Val Lys Arg
 195 200 205
 Tyr Asn Trp Thr Tyr Val Ser Ala Val His Thr Glu Gly Asn Tyr Gly
 210 215 220
 Glu Ser Gly Met Glu Ala Phe Lys Asp Met Ser Ala Lys Glu Gly Ile
 225 230 235 240
 Cys Ile Ala His Ser Tyr Lys Ile Tyr Ser Asn Ala Gly Glu Gln Ser
 245 250 255
 Phe Asp Lys Leu Leu Lys Lys Leu Thr Ser His Leu Pro Lys Ala Arg
 260 265 270
 Val Val Ala Cys Phe Cys Glu Gly Met Thr Val Arg Gly Leu Leu Met
 275 280 285
 Ala Met Arg Arg Leu Gly Leu Ala Gly Glu Phe Leu Leu Leu Gly Ser
 290 295 300
 Asp Gly Trp Ala Asp Arg Tyr Asp Val Thr Asp Gly Tyr Gln Arg Glu
 305 310 315 320
 Ala Val Gly Gly Ile Thr Ile Lys Leu Gln Ser Pro Asp Val Lys Trp
 325 330 335
 Phe Asp Asp Tyr Tyr Leu Lys Leu Arg Pro Glu Thr Asn His Arg Asn
 340 345 350
 Pro Trp Phe Gln Glu Phe Trp Gln His Arg Phe Gln Cys Arg Leu Glu
 355 360 365
 Gly Phe Pro Gln Glu Asn Ser Lys Tyr Asn Lys Thr Cys Asn Ser Ser
 370 375 380
 Leu Thr Leu Lys Thr His His Val Gln Asp Ser Lys Met Gly Phe Val
 385 390 395 400
 Ile Asn Ala Ile Tyr Ser Met Ala Tyr Gly Leu His Asn Met Gln Met
 405 410 415
 Ser Leu Cys Pro Gly Tyr Ala Gly Leu Cys Asp Ala Met Lys Pro Ile
 420 425 430

FIGURE 17(A)

Asp Gly Arg Lys Leu Leu Glu Ser Leu Met Lys Thr Asn Phe Thr Gly
 435 440 445
 Val Ser Gly Asp Thr Ile Leu Phe Asp Glu Asn Gly Asp Ser Pro Gly
 450 455 460
 Arg Tyr Glu Ile Met Asn Phe Lys Glu Met Gly Lys Asp Tyr Phe Asp
 465 470 475 480
 Tyr Ile Asn Val Gly Ser Trp Asp Asn Gly Glu Leu Lys Met Asp Asp
 485 490 495
 Asp Glu Val Trp Ser Lys Lys Ser Asn Ile Ile Arg Ser Val Cys Ser
 500 505 510
 Glu Pro Cys Glu Lys Gly Gln Ile Lys Val Ile Arg Lys Gly Glu Val
 515 520 525
 Ser Cys Cys Trp Thr Cys Thr Pro Cys Lys Glu Asn Glu Tyr Val Phe
 530 535 540
 Asp Glu Tyr Thr Cys Lys Ala Cys Gln Leu Gly Ser Trp Pro Thr Asp
 545 550 555 560
 Asp Leu Thr Gly Cys Asp Leu Ile Pro Val Gln Tyr Leu Arg Trp Gly
 565 570 575
 Asp Pro Glu Pro Ile Ala Ala Val Val Phe Ala Cys Leu Gly Leu Leu
 580 585 590
 Ala Thr Leu Phe Val Thr Val Val Phe Ile Ile Tyr Arg Asp Thr Pro
 595 600 605
 Val Val Lys Ser Ser Ser Arg Glu Leu Cys Tyr Ile Ile Leu Ala Gly
 610 615 620
 Ile Cys Leu Gly Tyr Leu Cys Thr Phe Cys Leu Ile Ala Lys Pro Lys
 625 630 635 640
 Gln Ile Tyr Cys Tyr Leu Gln Arg Ile Gly Ile Gly Leu Ser Pro Ala
 645 650 655
 Met Ser Tyr Ser Ala Leu Val Thr Lys Thr Asn Arg Ile Ala Arg Ile
 660 665 670
 Leu Ala Gly Ser Lys Lys Lys Ile Cys Thr Lys Lys Pro Arg Phe Met
 675 680 685
 Ser Ala Cys Ala Gln Leu Val Ile Ala Phe Ile Leu Ile Cys Ile Gln
 690 695 700
 Leu Gly Ile Ile Val Ala Leu Phe Ile Met Glu Pro Pro Asp Ile Met
 705 710 715 720
 His Asp Tyr Pro Ser Ile Arg Glu Val Tyr Leu Ile Cys Asn Thr Thr
 725 730 735
 Asn Leu Gly Val Val Thr Pro Leu Gly Tyr Asn Gly Leu Ile Leu
 740 745 750
 Ser Cys Thr Phe Tyr Ala Phe Lys Thr Arg Asn Val Pro Ala Asn Phe
 755 760 765
 Asn Glu Ala Lys Tyr Ile Ala Phe Thr Met Tyr Thr Thr Cys Ile Ile
 770 775 780
 Trp Leu Ala Phe Val Pro Ile Tyr Phe Gly Ser Asn Tyr Lys Ile Ile
 785 790 795 800
 Thr Met Cys Phe Ser Val Ser Leu Ser Ala Thr Val Ala Leu Gly Cys
 805 810 815
 Met Phe Val Pro Lys Val Tyr Ile Ile Leu Ala Lys Pro Glu Arg Asn
 820 825 830
 Val Arg Ser Ala Phe Thr Thr Ser Thr Val Val Arg Met His Val Gly
 835 840 845
 Asp Gly Lys Ser Ser Ser Ala Ala Ser Arg Ser Ser Ser Leu Val Asn
 850 855 860
 Leu Trp Lys Arg Arg Gly Ser Ser Gly Glu Thr Leu Arg Tyr Lys Asp

FIGURE 17(B)

8765										870										875										880													
Arg	Arg	Leu	Ala	Gln	His	Lys	Ser	Glu	Ile	Glu	Cys	Phe	Thr	Pro	Lys	Gly	Asn	Gly	Gly	Arg	Ala	Thr	Met	Ser	Ser	Ser	Asn	Gly	Leu	Val	Thr	Trp	Ala	Gln	Asn	Glu	Lys	Ser	Ser	Arg	Gly	Gln	His
885										890										895																							
Gly	Ser	Met	Gly	Asn	Gly	Gly	Arg	Ala	Thr	Met	Ser	Ser	Ser	Ser	Asn	Gly																											
900										905										910																							
Lys	Ser	Val	Thr	Trp	Ala	Gln	Asn	Glu	Lys	Ser	Ser	Arg	Gly	Gln	His																												
915										920										925																							
Leu	Trp	Gln	Arg	Leu	Ser	Ile	His	Ile	Asn	Lys	Lys	Glu	Asn	Pro	Asn																												
930										935										940																							
Gln	Thr	Ala	Val	Ile	Lys	Pro	Phe	Pro	Lys	Ser	Thr	Glu	Ser	Arg	Gly																												
945										950										955																							
Leu	Gly	Ala	Gly	Ala	Gly	Ala	Gly	Gly	Ser	Ala	Gly	Gly	Val	Gly	Ala																												
965										970										975																							
Thr	Gly	Gly	Ala	Gly	Cys	Ala	Gly	Ala	Gly	Pro	Gly	Gly	Pro	Glu	Ser																												
980										985										990																							
Pro	Asp	Ala	Gly	Pro	Lys	Ala	Leu	Tyr	Asp	Val	Ala	Glu	Ala	Glu	Glu																												
995										1000										1005																							
His	Phe	Pro	Ala	Pro	Ala	Arg	Pro	Arg	Ser	Pro	Ser	Pro	Ile	Ser	Thr																												
1010										1015										1020																							
Leu	Ser	His	Arg	Ala	Gly	Ser	Ala	Ser	Arg	Thr	Asp	Asp	Asp	Val	Pro																												
1025										1030										1035																							
Ser	Leu	His	Ser	Glu	Pro	Val	Ala	Arg	Ser	Ser	Ser	Ser	Gln	Gly	Ser																												
1045										1050										1055																							
Leu	Met	Glu	Gln	Ile	Ser	Ser	Val	Val	Thr	Arg	Phe	Thr	Ala	Asn	Ile																												
1060										1065										1070																							
Ser	Glu	Leu	Asn	Ser	Met	Met	Leu	Ser	Thr	Ala	Ala	Pro	Ser	Pro	Gly																												
1075										1080										1085																							
Val	Gly	Ala	Pro	Leu	Cys	Ser	Ser	Tyr	Leu	Ile	Pro	Lys	Glu	Ile	Gln																												
1090										1095										1100																							
Leu	Pro	Thr	Thr	Met	Thr	Thr	Phe	Ala	Glu	Ile	Gln	Pro	Leu	Pro	Ala																												
1105										1110										1115																							
Ile	Glu	Val	Thr	Gly	Gly	Ala	Gln	Pro	Ala	Ala	Gly	Ala	Gln	Ala	Ala																												
1125										1130										1135																							
Gly	Asp	Ala	Ala	Arg	Glu	Ser	Pro	Ala	Ala	Gly	Pro	Glu	Ala	Ala	Ala																												
1140										1145										1150																							
Ala	Lys	Pro	Asp	Leu	Glu	Glu	Leu	Val	Ala	Leu	Thr	Pro	Pro	Ser	Pro																												
1155										1160										1165																							
Phe	Arg	Asp	Ser	Val	Asp	Ser	Gly	Ser	Thr	Thr	Pro	Asn	Ser	Pro	Val																												
1170										1175										1180																							
Ser	Glu	Ser	Ala	Leu	Cys	Ile	Pro	Ser	Ser	Pro	Lys	Tyr	Asp	Thr	Leu																												
1185										1190										1195																							
Ile	Ile	Arg	Asp	Tyr	Thr	Gln	Ser	Ser	Ser	Ser	Ser	Leu																															
1205										1210																																	

FIGURE 17(C)

SEQUENCE LISTING

(1) GENERAL INFORMATION

- 5 (i) APPLICANT: Johnson, Thomas E.
Sikela, James M.
Simpson, Victoria J.
Rikke, Brad A.
- 10 (ii) TITLE OF THE INVENTION: POLYNUCLEOTIDE AND POLYPEPTIDE
SEQUENCES ASSOCIATED WITH CNS DEPRESSANT SENSITIVITY AND
METHODS OF USE THEREOF
- 15 (iii) NUMBER OF SEQUENCES: 7
- (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: MORRISON & FOERSTER
(B) STREET: 755 PAGE MILL ROAD
(C) CITY: Palo Alto
20 (D) STATE: CA
(E) COUNTRY: USA
(F) ZIP: 94304-1018
- 25 (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0
- 30 (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: To Be Assigned
(B) FILING DATE: Herewith
(C) CLASSIFICATION:
- 35 (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 60/073,190
(B) FILING DATE: January 30, 1998
- 40 (viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Catherine, Polizzi M
(B) REGISTRATION NUMBER: 40,130
(C) REFERENCE/DOCKET NUMBER: 34293-20003.00
- 45 (ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 650-813-5600
(B) TELEFAX: 650-494-0792
(C) TELEX: 706141
- 50 (2) INFORMATION FOR SEQ ID NO:1:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3591 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
55 (D) TOPOLOGY: linear
- (ix) FEATURE:
(A) NAME/KEY: Coding Sequence
60 (B) LOCATION: 1...3591
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

5	ATG CTC CTT CTG TTG ATT CTG TCA GTC CTA CTT CTG AAA GAA GAT GTA Met Val Leu Leu Leu Ile Leu Ser Val Leu Leu Leu Lys Glu Asp Val 1 5 10 15	48
10	CGA GGA AGT GCA CAG TCC AGT GAG AGG AGG GTG GTG GCT CAC ATG CCA Arg Gly Ser Ala Gln Ser Ser Glu Arg Arg Val Val Ala His Met Pro 20 25 30	96
15	GGT GAC ATT ATT ATT GGA GGT CTC TTC TCG GTC CAC CAC CAA CCA ACT Gly Asp Ile Ile Ile Gly Ala Leu Phe Ser Val His His Gln Pro Thr 35 40 45	144
20	GTG GAC AAA GTT CAT GAG AGG AAG TGT GGG GCA GTC CGT GAG CAG TAT Val Asp Lys Val His Glu Arg Lys Cys Gly Ala Val Arg Glu Gln Tyr 50 55 60	192
25	GGC ATT CAG AGA GTG GAA GCC ATG CTG CAT ACC TTG GAA AGC ATC AAT Gly Ile Gln Arg Val Glu Ala Met Leu His Thr Leu Glu Arg Ile Asn 65 70 75 80	240
30	TCA GAT CCC ACA CTC TTG CCC AAC ATC ACA CTA GGC TGT GAG ATA AGA Ser Asp Pro Thr Leu Leu Pro Asn Ile Thr Leu Gly Cys Glu Ile Arg 85 90 95	288
35	GAT TCC TGC TGG CAT TCT GCT GTG GCC CTA GAG CAA AGC ATT GAG TTT Asp Ser Cys Trp His Ser Ala Val Ala Leu Glu Gln Ser Ile Glu Phe 100 105 110	336
40	ATA AGG GAT TCC CTC ATC TCT TCG GAA GAG GAA GAA GGC TTG GTA CGC Ile Arg Asp Ser Leu Ile Ser Ser Glu Glu Glu Glu Gly Leu Val Arg 115 120 125	384
45	TGT GTA GAT GGC TCT TCT TCC TTC CGC TCC AAG AAA CCC ATA GTG GGA Cys Val Asp Gly Ser Ser Ser Phe Arg Ser Lys Lys Pro Ile Val Gly 130 135 140	432
50	GTC ATT GGG CCT GGC TCG AGT TCT GTG GCC ATT CAA GTT CAG AAC TTG Val Ile Gly Pro Gly Ser Ser Ser Val Ala Ile Gln Val Gln Asn Leu 145 150 155 160	480
55	CTC CAG CTT TTC AAC ATA CCT CAG ATT GCT TAC TCT GCA ACT AGC ATG Leu Gln Leu Phe Asn Ile Pro Gln Ile Ala Tyr Ser Ala Thr Ser Met 165 170 175	528
60	GAT TTG AGT GAC AAG ACT CTA TTC AAG TAC TTC ATG AGG GTT GTA CCT Asp Leu Ser Asp Lys Thr Leu Phe Lys Tyr Phe Met Arg Val Val Pro 180 185 190	576
65	TCG GAT GCC CAG CAA GCC CGA GCC ATG GTA GAC ATA GTG AAG AGA TAC Ser Asp Ala Gln Gln Ala Arg Ala Met Val Asp Ile Val Lys Arg Tyr 195 200 205	624
70	AAC TGG ACT TAT GTC TCA GGT GTG CAC ACA GAA GGC AAC TAT GGA GAA Asn Trp Thr Tyr Val Ser Ala Val His Thr Glu Gly Asn Tyr Gly Glu 210 215 220	672
75	AGT GGG ATG GAG GCT TTC AAA GAT ATG TCA GCG AAG GAA GGG ATT TGC Ser Gly Met Glu Ala Phe Lys Asp Met Ser Ala Lys Glu Gly Ile Cys 225 230 235 240	720

	225	230	235	240	
5	ATC GGC CAC TCT TAC AAA ATC TAC AGC AAT GCT GGG GAA CAG AGC TTT Ile Ala His Ser Tyr Lys Ile Tyr Ser Asn Ala Gly Glu Gln Ser Phe 245 250 255	768			
10	GAC AAG CTG TTG AAA AAG CTC AGA AGT CAT TTA CCT AAA GGC CGG GTG Asp Lys Leu Leu Lys Lys Leu Arg Ser His Leu Pro Lys Ala Arg Val 260 265 270	816			
15	GTA GGC TGC TTC TGT GAA GGC ATG ACA GTT CGA GGT CTG CTC ATG GCC Val Ala Cys Phe Cys Glu Gly Met Thr Val Arg Gly Leu Leu Met Ala 275 280 285	864			
20	ATG AGA CGC TTG GGT CTA GCA GGG GAA TTT CTA CTT CTG GGC AGT GAT Met Arg Arg Leu Gly Leu Ala Gly Glu Phe Leu Leu Leu Gly Ser Asp 290 295 300	912			
25	GGC TGG GCT GAC AGG TAT GAC GTG ACA GAT GGG TAT CAG CGA GAA GCT Gly Trp Ala Asp Arg Tyr Asp Val Thr Asp Gly Tyr Gln Arg Glu Ala 305 310 315 320	960			
30	CTC GGT GGG ATT ACA ATC AAG CTC CAG TCT CCT GAT GTC AAG TGG TTT Val Gly Gly Ile Thr Ile Lys Leu Gln Ser Pro Asp Val Lys Trp Phe 325 330 335	1008			
35	GAT GAT TAT TAT CTG AAG CTC CGG CCA GAA ACA AAC CTC AGA AAC CCT Asp Asp Tyr Tyr Leu Lys Leu Arg Pro Glu Thr Asn Leu Arg Asn Pro 340 345 350	1056			
40	TGG TTT CAA GAA TTT TGG CAG CAT CGT TTT CAG TGC CGG CTA GAA GGG Trp Phe Gln Glu Phe Trp Gln His Arg Phe Gln Cys Arg Leu Glu Gly 355 360 365	1104			
45	TTT GCA CAG GAG AAC AGC AAG TAC AAC AAG ACT TGC AAC AGT TCT CTA Phe Ala Gln Glu Asn Ser Lys Tyr Asn Lys Thr Cys Asn Ser Ser Leu 370 375 380	1152			
50	ACT CTG AGA ACG CAT CAT GTT CAA GAT TCC AAA ATG GGA TTT GTG ATC Thr Leu Arg Thr His His Val Gln Asp Ser Lys Met Gly Phe Val Ile 385 390 395 400	1200			
55	AAT GCA ATC TAT TCT ATG GCT TAT GGG CTC CAC AAC ATG CAG ATG TCC Asn Ala Ile Tyr Ser Met Ala Tyr Gly Leu His Asn Met Gln Met Ser 405 410 415	1248			
60	CTG TGT CCA GGC TAT GCA GGC CTC TGT GAT GCA ATG AAG CCA ATT GAT Leu Cys Pro Gly Tyr Ala Gly Leu Cys Asp Ala Met Lys Pro Ile Asp 420 425 430	1296			
65	GGG CGG AAA CTT TTG GAC TCC CTG ATG AAA ACC AAC TTT ACT GGA GTT Gly Arg Lys Leu Leu Asp Ser Leu Met Lys Thr Asn Phe Thr Gly Val 435 440 445	1344			
70	TCC GGA GAT ATG ATT CTA TTT GAT GAA AAT GGA GAC TCT CCA GGA AGG Ser Gly Asp Met Ile Leu Phe Asp Glu Asn Gly Asp Ser Pro Gly Arg 450 455 460	1392			
75	TAT GAA ATA ATG AAT TTC AAG GAA ATG GGA AAA GAT TAT TTT GAT TAC Tyr Glu Ile Met Asn Phe Lys Glu Met Gly Lys Asp Tyr Phe Asp Tyr 465 470 475 480	1440			

5	ATC AAT GTT GGA AGT TGG GAC AAT GGG GAA TTA AAA ATG GAT GAT GAT	1488
	Ile Asn Val Gly Ser Trp Asp Asn Gly Glu Leu Lys Met Asp Asp Asp 485 490 495	
10	GAA GTG TGG TCC AAG AAA AAT AAC ATC ATC AGA TCT GTG TGC AGT GAA	1536
	Glu Val Trp Ser Lys Lys Asn Asn Ile Ile Arg Ser Val Cys Ser Glu 500 505 510	
15	CCG TGT GAG AAA GGA CAG ATA AAG GTG ATC CCG AAG GGA GAA GTC AGC	1584
	Pro Cys Glu Lys Gly Gln Ile Lys Val Ile Arg Lys Gly Glu Val Ser 515 520 525	
20	TGT TGT TGG ACC TGC ACA CCT TGT AAG GAG AAT GAG TAT GTT TTT GAT	1632
	Cys Cys Trp Thr Cys Thr Pro Cys Lys Glu Asn Glu Tyr Val Phe Asp 530 535 540	
25	GAG TAC ACC TGC AAG GCG TGC CAA CTG GGG TCC TGG CCC ACT GAC GAC	1680
	Glu Tyr Thr Cys Lys Ala Cys Gln Leu Gly Ser Trp Pro Thr Asp Asp 545 550 555 560	
30	TTG ACA GGT TGT GAT TTG ATC CCA GTC CAG TAT CTT CGA TGG GGT GAC	1728
	Leu Thr Gly Cys Asp Leu Ile Pro Val Gln Tyr Leu Arg Trp Gly Asp 565 570 575	
35	CCT GAG CCC ATT GCA GCT GTG GTG TTT GCC TGC CTC GGT CTG CTA GCC	1776
	Pro Glu Pro Ile Ala Ala Val Val Phe Ala Cys Leu Gly Leu Leu Ala 580 585 590	
40	ACC CTC TTC GTT ACT GTA ATC TTC ATC ATT TAT CGG GAC ACT CCA GTG	1824
	Thr Leu Phe Val Thr Val Ile Phe Ile Ile Tyr Arg Asp Thr Pro Val 595 600 605	
45	GTC AAG TCT TCC AGC AGG GAA CTC TGC TAC ATT ATC CTT GCT GGC ATC	1872
	Val Lys Ser Ser Ser Arg Glu Leu Cys Tyr Ile Ile Leu Ala Gly Ile 610 615 620	
50	TGC CTG GGT TAC TTG TGT ACC TTC TGC CTC ATT GCA AAG CCC AAA CAG	1920
	Cys Leu Gly Tyr Leu Cys Thr Phe Cys Leu Ile Ala Lys Pro Lys Gln 625 630 635 640	
55	ATT TAC TGC TAC CTT CAG AGA ATT GGC ATC GGT CTC TCT CCA GCC ATG	1968
	Ile Tyr Cys Tyr Leu Gln Arg Ile Gly Ile Gly Leu Ser Pro Ala Met 645 650 655	
60	AGC TAC TCA GCC CTT GTA ACG AAG ACC AAC CGT ATT GCA AGG ATT CTA	2016
	Ser Tyr Ser Ala Leu Val Thr Lys Thr Asn Arg Ile Ala Arg Ile Leu 660 665 670	
65	GCT GGC AGC AAG AAG AAG ATC TGT ACC AAA AAG CCC AGA TTC ATG AGC	2064
	Ala Gly Ser Lys Lys Lys Ile Cys Thr Lys Lys Pro Arg Phe Met Ser 675 680 685	
70	GCC TGT GCT CAG TTA GTG ATC GCT TTC ATT CTC ATC TGT ATA CAG TTG	2112
	Ala Cys Ala Gln Leu Val Ile Ala Phe Ile Leu Ile Cys Ile Gln Leu 690 695 700	
75	GST ATT ATT GTG GCC CTC TTT ATC ATG GAG CCT CCG GAT ATA ATG CAT	2160
	Gly Ile Ile Val Ala Leu Phe Ile Met Glu Pro Pro Asp Ile Met His 705 710 715 720	

	GAC TAT CCA AGC ATC CGA GAA GTC TAC TTG ATT TGT AAC ACC ACC AAC	2208
	Asp Tyr Pro Ser Ile Arg Glu Val Tyr Leu Ile Cys Asn Thr Thr Asn	
	725 730 735	
5	CTA GGG GTT GTC ACT CCT CTT GSA TAC AAT GGA TTA TTG ATT TTG AGT	2256
	Leu Gly Val Val Thr Pro Leu Gly Tyr Asn Gly Leu Leu Ile Leu Ser	
	740 745 750	
10	TGC ACA TTC TAT GCG TTC AAG ACC AGA AAT GTT CCA GCC AAC TTT AAC	2304
	Cys Thr Phe Tyr Ala Phe Lys Thr Arg Asn Val Pro Ala Asn Phe Asn	
	755 760 765	
15	GAG GCC AAA TAT ATT GCT TTC ACC ATG TAC ACA ACC TGC ATC ATA TGG	2352
	Glu Ala Lys Tyr Ile Ala Phe Thr Met Tyr Thr Thr Cys Ile Ile Trp	
	770 775 780	
20	CTG GCC TTT GTG CCT ATC TAC TTT GGC AGC AAC TAC AAA ATC ATC ACC	2400
	Leu Ala Phe Val Pro Ile Tyr Phe Gly Ser Asn Tyr Lys Ile Ile Thr	
	785 790 795 800	
	ATG TGT TTC TCA GTC AGC CTC AGT GCC ACA GTG GCC CTG GGT TGC ATG	2448
	Met Cys Phe Ser Val Ser Leu Ser Ala Thr Val Ala Leu Gly Cys Met	
	805 810 815	
25	TTT GTG CCG AAG GTG TAC ATC ATC CTA GCC AAA CCG GAG AGA AAT GTG	2496
	Phe Val Pro Lys Val Tyr Ile Ile Leu Ala Lys Pro Glu Arg Asn Val	
	820 825 830	
30	CGC AGC GCC TTC ACA ACC TCT ACA GTG GTG CGC ATG CAC GTA GGA GAT	2544
	Arg Ser Ala Phe Thr Thr Ser Thr Val Val Arg Met His Val Gly Asp	
	835 840 845	
35	GGC AAG TCA TCA TCC GCT GCC AGC AGA TCC AGC AGC CTA GTC AAC CTG	2592
	Gly Lys Ser Ser Ser Ala Ala Ser Arg Ser Ser Ser Leu Val Asn Leu	
	850 855 860	
40	TGG AAG AGG AGG GGC TCG TCT GGG GAA ACC CTA AGG TAC AAA GAC AGG	2640
	Trp Lys Arg Arg Gly Ser Ser Gly Glu Thr Leu Arg Tyr Lys Asp Arg	
	865 870 875 880	
	AGA CTG GCC CAG CAC AAG TCG GAA ATA GAG TGT TTC ACC CCC AAA GGG	2688
	Arg Leu Ala Gln His Lys Ser Glu Ile Glu Cys Phe Thr Pro Lys Gly	
	885 890 895	
45	AGT ATG GGG AAT GGT GGG AGA GCA ACA ATG AGC AGC TCC AAT GGA AAA	2736
	Ser Met Gly Asn Gly Gly Arg Ala Thr Met Ser Ser Ser Asn Gly Lys	
	900 905 910	
50	TCC GTG ACT TGG GCC CAG AAT GAG AAG AGC ACC CGG GGG CAG CAC CTG	2784
	Ser Val Thr Trp Ala Gln Asn Glu Lys Ser Thr Arg Gly Gln His Leu	
	915 920 925	
55	TGG CAG CGA CTG TCT GTC CAT ATC AAC AAG AAG GAG AAC CCC AAC CAG	2832
	Trp Gln Arg Leu Ser Val His Ile Asn Lys Lys Glu Asn Pro Asn Gln	
	930 935 940	
60	ACA GCA GTC ATC AAA CCC TTC CCC AAG AGC ACA GAG AGC CGC GGG CAG	2880
	Thr Ala Val Ile Lys Pro Phe Pro Lys Ser Thr Glu Ser Arg Gly Gln	
	945 950 955 960	
	GGT GCA GGG GCA GGT GGT GGC TCT GGC CCC GGT GCA GCT GGT GCT GGT	2928

	Gly Ala Gly Ala Gly Gly Gly Ser Gly Pro Gly Ala Ala Gly Ala Gly	
	965 970 975	
5	ACC GCA GGA TGC ACA GCG ACA GGC GGC CCA GAG CCA CCA GAC GCC GGC Ser Ala Gly Cys Thr Ala Thr Gly Gly Pro Glu Pro Pro Asp Ala Gly 980 985 990	2976
10	CCG AAG GCG CTT TAT GAT GTC GCA GAG GCA GAG GAG CGC TTC CCA GCG Pro Lys Ala Leu Tyr Asp Val Ala Glu Ala Glu Glu Arg Phe Pro Ala 995 1000 1005	3024
15	GGT GCC AGG CCG CGC TCG CCA TCG CCC ATC AGT ACG CTG AGC CAC CTG Ala Ala Arg Pro Arg Ser Pro Ser Pro Ile Ser Thr Leu Ser His Leu 1010 1015 1020	3072
	GGA GGC TCG GCG GGC CGC ACA GAC GAC GAC GCG CCG TCG CTG CAC TCG Ala Gly Ser Ala Gly Arg Thr Asp Asp Asp Ala Pro Ser Leu His Ser 1025 1030 1035 1040	3120
20	GAG ACC GCT GCA CGC AGC AGC TCA TCC CAG GGC TCG CTC ATG GAG CAG Glu Thr Ala Ala Arg Ser Ser Ser Ser Gln Gly Ser Leu Met Glu Gln 1045 1050 1055	3168
25	ATT AGC AGC GTG GTG ACG CGC TTC ACC GCC AAC ATC ACC GAG CTC AAC Ile Ser Ser Val Val Thr Arg Phe Thr Ala Asn Ile Thr Glu Leu Asn 1060 1065 1070	3216
30	TCC ATG ATG TTG TCC ACC GCG GCT GCG CCG GGG CCC CCT GGT ACC CCT Ser Met Met Leu Ser Thr Ala Ala Ala Pro Gly Pro Pro Gly Thr Pro 1075 1080 1085	3264
35	ATC TCC TCT TCC TAC CTG ATC CCC AAA GAG ATC CAG CTG CCC ACG ACC Ile Cys Ser Ser Tyr Leu Ile Pro Lys Glu Ile Gln Leu Pro Thr Thr 1090 1095 1100	3312
	ATG ACG ACC TTC GCA GAG ATC CAG CCT CTG CCG GCC ATC GAG GTG ACC Met Thr Thr Phe Ala Glu Ile Gln Pro Leu Pro Ala Ile Glu Val Thr 1105 1110 1115 1120	3360
40	GGA GGA GGT CAG CCG GCG ACA GGG CCA TCA CCT GCC CAA GAG ACG CCC Gly Gly Ala Gln Pro Ala Thr Gly Pro Ser Pro Ala Gln Glu Thr Pro 1125 1130 1135	3408
45	GCA GGA GGT GAA GCC GCC CCA GGA AAA CCG GAT CTG GAG GAG CTG GTG Ala Gly Ala Glu Ala Ala Pro Gly Lys Pro Asp Leu Glu Glu Leu Val 1140 1145 1150	3456
50	GCC CTC ACT CCA CCA TCG CCC TTC AGG GAC TCG GTG GAC TCG GGG AGC Ala Leu Thr Pro Pro Ser Pro Phe Arg Asp Ser Val Asp Ser Gly Ser 1155 1160 1165	3504
55	ACC ACC CCA AAC TCT CCA GTC TCC GAA TCG GCC CTC TGC ATC CCA TCC Thr Thr Pro Asn Ser Pro Val Ser Glu Ser Ala Leu Cys Ile Pro Ser 1170 1175 1180	3552
60	TCT CCC AAA TAT GAC ACT CTC ATC ATC AGA GAT TAC ACG Ser Pro Lys Tyr Asp Thr Leu Ile Ile Arg Asp Tyr Thr 1185 1190 1195	3591

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1197 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

	Met	Val	Leu	Leu	Leu	Ile	Leu	Ser	Val	Leu	Leu	Leu	Lys	Glu	Asp	Val
	1				5					10					15	
15	Arg	Gly	Ser	Ala	Gln	Ser	Ser	Glu	Arg	Arg	Val	Val	Ala	His	Met	Pro
				20					25					30		
	Gly	Asp	Ile	Ile	Ile	Gly	Ala	Leu	Phe	Ser	Val	His	His	Gln	Pro	Thr
			35					40					45			
20	Val	Asp	Lys	Val	His	Glu	Arg	Lys	Cys	Gly	Ala	Val	Arg	Glu	Gln	Tyr
		50					55					60				
	Gly	Ile	Gln	Arg	Val	Glu	Ala	Met	Leu	His	Thr	Leu	Glu	Arg	Ile	Asn
	65				70					75					80	
	Ser	Asp	Pro	Thr	Leu	Pro	Asn	Ile	Thr	Leu	Gly	Cys	Glu	Ile	Arg	
				85					90					95		
25	Asp	Ser	Cys	Trp	His	Ser	Ala	Val	Ala	Leu	Glu	Gln	Ser	Ile	Glu	Phe
				100					105					110		
	Ile	Arg	Asp	Ser	Leu	Ile	Ser	Ser	Glu	Glu	Glu	Glu	Gly	Leu	Val	Arg
			115					120					125			
30	Cys	Val	Asp	Gly	Ser	Ser	Ser	Phe	Arg	Ser	Lys	Lys	Pro	Ile	Val	Gly
		130						135				140				
	Val	Ile	Gly	Pro	Gly	Ser	Ser	Ser	Val	Ala	Ile	Gln	Val	Gln	Asn	Leu
	145				150					155					160	
	Leu	Gln	Leu	Phe	Asn	Ile	Pro	Gln	Ile	Ala	Tyr	Ser	Ala	Thr	Ser	Met
				165						170					175	
35	Asp	Leu	Ser	Asp	Lys	Thr	Leu	Phe	Lys	Tyr	Phe	Met	Arg	Val	Val	Pro
				180					185					190		
	Ser	Asp	Ala	Gln	Gln	Ala	Arg	Ala	Met	Val	Asp	Ile	Val	Lys	Arg	Tyr
			195					200					205			
40	Asn	Trp	Thr	Tyr	Val	Ser	Ala	Val	His	Thr	Glu	Gly	Asn	Tyr	Gly	Glu
		210					215					220				
	Ser	Gly	Met	Glu	Ala	Phe	Lys	Asp	Met	Ser	Ala	Lys	Glu	Gly	Ile	Cys
	225				230						235				240	
	Ile	Ala	His	Ser	Tyr	Lys	Ile	Tyr	Ser	Asn	Ala	Gly	Glu	Gln	Ser	Phe
				245						250				255		
45	Asp	Lys	Leu	Leu	Lys	Lys	Leu	Arg	Ser	His	Leu	Pro	Lys	Ala	Arg	Val
			260						265					270		
	Val	Ala	Cys	Phe	Cys	Glu	Gly	Met	Thr	Val	Arg	Gly	Leu	Leu	Met	Ala
		275						280					285			
50	Met	Arg	Arg	Leu	Gly	Leu	Ala	Gly	Glu	Phe	Leu	Leu	Leu	Gly	Ser	Asp
		290					295					300				
	Gly	Trp	Ala	Asp	Arg	Tyr	Asp	Val	Thr	Asp	Gly	Tyr	Gln	Arg	Glu	Ala
	305				310						315				320	
	Val	Gly	Gly	Ile	Thr	Ile	Lys	Leu	Gln	Ser	Pro	Asp	Val	Lys	Trp	Phe
				325						330				335		
55	Asp	Asp	Tyr	Tyr	Leu	Lys	Leu	Arg	Pro	Glu	Thr	Asn	Leu	Arg	Asn	Pro
			340					345					350			
	Trp	Phe	Gln	Glu	Phe	Trp	Gln	His	Arg	Phe	Gln	Cys	Arg	Leu	Glu	Gly
		355					360					365				
60	Phe	Ala	Gln	Glu	Asn	Ser	Lys	Tyr	Asn	Lys	Thr	Cys	Asn	Ser	Ser	Leu
		370				375					380					
	Thr	Leu	Arg	Thr	His	His	Val	Gln	Asp	Ser	Lys	Met	Gly	Phe	Val	Ile

	385					390					395					400
	Asn	Ala	Ile	Tyr	Ser	Met	Ala	Tyr	Gly	Leu	His	Asn	Met	Gln	Met	Ser
					405					410					415	
5	Leu	Cys	Pro	Gly	Tyr	Ala	Gly	Leu	Cys	Asp	Ala	Met	Lys	Pro	Ile	Asp
				420					425					430		
	Gly	Arg	Lys	Leu	Leu	Asp	Ser	Leu	Met	Lys	Thr	Asn	Phe	Thr	Gly	Val
			435					440					445			
	Ser	Gly	Asp	Met	Ile	Leu	Phe	Asp	Glu	Asn	Gly	Asp	Ser	Pro	Gly	Arg
		450					455					460				
10	Tyr	Glu	Ile	Met	Asn	Phe	Lys	Glu	Met	Gly	Lys	Asp	Tyr	Phe	Asp	Tyr
	465					470					475				480	
	Ile	Asn	Val	Gly	Ser	Trp	Asp	Asn	Gly	Glu	Leu	Lys	Met	Asp	Asp	Asp
				485						490				495		
	Glu	Val	Trp	Ser	Lys	Lys	Asn	Asn	Ile	Ile	Arg	Ser	Val	Cys	Ser	Glu
15				500					505					510		
	Pro	Cys	Glu	Lys	Gly	Gln	Ile	Lys	Val	Ile	Arg	Lys	Gly	Glu	Val	Ser
			515					520					525			
	Cys	Cys	Trp	Thr	Cys	Thr	Pro	Cys	Lys	Glu	Asn	Glu	Tyr	Val	Phe	Asp
		530					535					540				
20	Glu	Tyr	Thr	Cys	Lys	Ala	Cys	Gln	Leu	Gly	Ser	Trp	Pro	Thr	Asp	Asp
	545					550					555				560	
	Leu	Thr	Gly	Cys	Asp	Leu	Ile	Pro	Val	Gln	Tyr	Leu	Arg	Trp	Gly	Asp
				565					570					575		
25	Pro	Glu	Pro	Ile	Ala	Ala	Val	Val	Phe	Ala	Cys	Leu	Gly	Leu	Leu	Ala
				580					585					590		
	Thr	Leu	Phe	Val	Thr	Val	Ile	Phe	Ile	Ile	Tyr	Arg	Asp	Thr	Pro	Val
			595					600					605			
	Val	Lys	Ser	Ser	Ser	Arg	Glu	Leu	Cys	Tyr	Ile	Ile	Leu	Ala	Gly	Ile
		610					615					620				
30	Cys	Leu	Gly	Tyr	Leu	Cys	Thr	Phe	Cys	Leu	Ile	Ala	Lys	Pro	Lys	Gln
	625					630					635				640	
	Ile	Tyr	Cys	Tyr	Leu	Gln	Arg	Ile	Gly	Ile	Gly	Leu	Ser	Pro	Ala	Met
				645					650					655		
35	Ser	Tyr	Ser	Ala	Leu	Val	Thr	Lys	Thr	Asn	Arg	Ile	Ala	Arg	Ile	Leu
				660					665					670		
	Ala	Gly	Ser	Lys	Lys	Lys	Ile	Cys	Thr	Lys	Lys	Pro	Arg	Phe	Met	Ser
			675					680					685			
	Ala	Cys	Ala	Gln	Leu	Val	Ile	Ala	Phe	Ile	Leu	Ile	Cys	Ile	Gln	Leu
		690					695					700				
40	Gly	Ile	Ile	Val	Ala	Leu	Phe	Ile	Met	Glu	Pro	Pro	Asp	Ile	Met	His
	705					710					715				720	
	Asp	Tyr	Pro	Ser	Ile	Arg	Glu	Val	Tyr	Leu	Ile	Cys	Asn	Thr	Thr	Asn
				725					730					735		
45	Leu	Gly	Val	Val	Thr	Pro	Leu	Gly	Tyr	Asn	Gly	Leu	Leu	Ile	Leu	Ser
				740					745					750		
	Cys	Thr	Phe	Tyr	Ala	Phe	Lys	Thr	Arg	Asn	Val	Pro	Ala	Asn	Phe	Asn
			755					760					765			
	Glu	Ala	Lys	Tyr	Ile	Ala	Phe	Thr	Met	Tyr	Thr	Thr	Cys	Ile	Ile	Trp
		770					775					780				
50	Leu	Ala	Phe	Val	Pro	Ile	Tyr	Phe	Gly	Ser	Asn	Tyr	Lys	Ile	Ile	Thr
	785					790					795				800	
	Met	Cys	Phe	Ser	Val	Ser	Leu	Ser	Ala	Thr	Val	Ala	Leu	Gly	Cys	Met
				805					810					815		
	Phe	Val	Pro	Lys	Val	Tyr	Ile	Ile	Leu	Ala	Lys	Pro	Glu	Arg	Asn	Val
55				820					825					830		
	Arg	Ser	Ala	Phe	Thr	Thr	Ser	Thr	Val	Val	Arg	Met	His	Val	Gly	Asp
			835					840					845			
	Gly	Lys	Ser	Ser	Ser	Ala	Ala	Ser	Arg	Ser	Ser	Ser	Leu	Val	Asn	Leu
		850					855					860				
60	Trp	Lys	Arg	Arg	Gly	Ser	Ser	Gly	Glu	Thr	Leu	Arg	Tyr	Lys	Asp	Arg
	865					870					875				880	

Arg Leu Ala Gln His Lys Ser Glu Ile Glu Cys Phe Thr Pro Lys Gly
 885 890 895
 Ser Met Gly Asn Gly Gly Arg Ala Thr Met Ser Ser Ser Asn Gly Lys
 900 905 910
 5 Ser Val Thr Trp Ala Gln Asn Glu Lys Ser Thr Arg Gly Gln His Leu
 915 920 925
 Trp Gln Arg Leu Ser Val His Ile Asn Lys Lys Glu Asn Pro Asn Gln
 930 935 940
 10 Thr Ala Val Ile Lys Pro Phe Pro Lys Ser Thr Glu Ser Arg Gly Gln
 945 950 955 960
 Gly Ala Gly Ala Gly Gly Gly Ser Gly Pro Gly Ala Ala Gly Ala Gly
 965 970 975
 Ser Ala Gly Cys Thr Ala Thr Gly Gly Pro Glu Pro Pro Asp Ala Gly
 980 985 990
 15 Pro Lys Ala Leu Tyr Asp Val Ala Glu Ala Glu Glu Arg Phe Pro Ala
 995 1000 1005
 Ala Ala Arg Pro Arg Ser Pro Ser Pro Ile Ser Thr Leu Ser His Leu
 1010 1015 1020
 20 Ala Gly Ser Ala Gly Arg Thr Asp Asp Asp Ala Pro Ser Leu His Ser
 025 1030 1035 1040
 Glu Thr Ala Ala Arg Ser Ser Ser Ser Gln Gly Ser Leu Met Glu Gln
 1045 1050 1055
 Ile Ser Ser Val Val Thr Arg Phe Thr Ala Asn Ile Thr Glu Leu Asn
 1060 1065 1070
 25 Ser Met Met Leu Ser Thr Ala Ala Ala Pro Gly Pro Pro Gly Thr Pro
 1075 1080 1085
 Ile Cys Ser Ser Tyr Leu Ile Pro Lys Glu Ile Gln Leu Pro Thr Thr
 1090 1095 1100
 30 Met Thr Thr Phe Ala Glu Ile Gln Pro Leu Pro Ala Ile Glu Val Thr
 105 1110 1115 1120
 Gly Gly Ala Gln Pro Ala Thr Gly Pro Ser Pro Ala Gln Glu Thr Pro
 1125 1130 1135
 Ala Gly Ala Glu Ala Ala Pro Gly Lys Pro Asp Leu Glu Glu Leu Val
 1140 1145 1150
 35 Ala Leu Thr Pro Pro Ser Pro Phe Arg Asp Ser Val Asp Ser Gly Ser
 1155 1160 1165
 Thr Thr Pro Asn Ser Pro Val Ser Glu Ser Ala Leu Cys Ile Pro Ser
 1170 1175 1180
 40 Ser Pro Lys Tyr Asp Thr Leu Ile Ile Arg Asp Tyr Thr
 185 1190 1195

(2) INFORMATION FOR SEQ ID NO:3:

45 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 100 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

50 (ix) FEATURE:

(A) NAME/KEY: Coding Sequence
 (B) LOCATION: 3...98
 (D) OTHER INFORMATION:

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

60 AG GTA CAA AGA CAG GAG ACT GGC CCA GCA CAA GTC GGA AAT AGA GTG
 Val Gln Arg Gln Glu Thr Gly Pro Ala Gln Val Gly Asn Arg Val
 1 5 10 15

47

TTT CAC CCC CAA AGG GAG TAT GGG GAA TGG TGG GAG AGC AAC AAT GAG 95
 Phe His Pro Gln Arg Glu Tyr Gly Glu Trp Trp Glu Ser Asn Asn Glu
 20 25 30

5 CAG GT 100
 Gln

(2) INFORMATION FOR SEQ ID NO:4:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 32 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: protein
 (v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

20 Val Gln Arg Gln Glu Thr Gly Pro Ala Gln Val Gly Asn Arg Val Phe
 1 5 10 15
 His Pro Gln Arg Glu Tyr Gly Glu Trp Trp Glu Ser Asn Asn Glu Gln
 20 25 30

25 (2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 3918 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

35 (ix) FEATURE:

(A) NAME/KEY: Coding Sequence
 (B) LOCATION: 268...3780
 (D) OTHER INFORMATION:

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TCAGACATCT CTTGAGAAAC TCAGCCTAGT TTATCCAGCT GTTTGTGCCA CATATGTTCA 60
 TAAAATCTAG TTATGAATAT TCTGATCGTA TGGATGCACC TAACTCGACA GTTCTG GGG 120
 ACTTTGCTAT CTTT TAGAGA ATATATCTAA ATTGCTGACT AATTCCTCAA TGCGCCACTC 180
 AACGCAGGGC ATCATTG GGC TGTACCTATT GGAGCTCTGA GTTCCTCCA TCATGATATC 240
 TTTATTAGCT TGAATTCCTT TCCTAAA ATG GTC CTT CTG TTG ATC CTG TCA GTC 294
 Met Val Leu Leu Leu Ile Leu Ser Val
 1 5

50 CTA CTT CTG AAA GAA GAT GTA CGA GGG AGT GCA CAG TCC AGT GAG AGG 342
 Leu Leu Leu Lys Glu Asp Val Arg Gly Ser Ala Gln Ser Ser Glu Arg
 10 15 20 25

55 AGG GTG GTG GCT CAC ATG CCA GGT GAC ATC ATT ATT GGA GCT CTC TTC 390
 Arg Val Val Ala His Met Pro Gly Asp Ile Ile Ile Gly Ala Leu Phe
 30 35 40

60 TCT GTC CAC CAC CAA CCA ACT GTG GAC AAA GTT CAT GAG AGG AAG TGT 438
 Ser Val His His Gln Pro Thr Val Asp Lys Val His Glu Arg Lys Cys
 45 50 55

	GGT GCA GTT GGT GAG CAA TAT GGG ATT CAG AGA GTG GAG GCC ATG CTG	486
	Gly Ala Val Arg Glu Gln Tyr Gly Ile Gln Arg Val Glu Ala Met Leu	
	60 65 70	
5	CAT ACC TTG GAA AGG ATC AAC TCA GAT CCC ACA CTC TTG CCC AAC ATC	534
	His Thr Leu Glu Arg Ile Asn Ser Asp Pro Thr Leu Leu Pro Asn Ile	
	75 80 85	
10	ACA CTT GGC TGT GAG ATC AGA GAT TCC TGC TGG CAT TCC GCT GTG GCC	582
	Thr Leu Gly Cys Glu Ile Arg Asp Ser Cys Trp His Ser Ala Val Ala	
	90 95 100 105	
15	CTA GAA CAA AGC ATT GAG TTT ATA AGG GAT TCC CTC ATC TCT TCA GAA	630
	Leu Glu Gln Ser Ile Glu Phe Ile Arg Asp Ser Leu Ile Ser Ser Glu	
	110 115 120	
20	GAG GAG GAA GGT TTG GTA CGC TGT GTA GAT GGA TCT TCT TCC TTC CGC	678
	Glu Glu Glu Gly Leu Val Arg Cys Val Asp Gly Ser Ser Ser Phe Arg	
	125 130 135	
25	TCC AAG AAA CCC ATA GTG GGA GTC ATT GGG CCT GGC TCG AGT TCT GTG	726
	Ser Lys Lys Pro Ile Val Gly Val Ile Gly Pro Gly Ser Ser Ser Val	
	140 145 150	
30	GCC ATT CAA GTC CAG AAC TTA CTC CAG CTT TTC AAC ATA CCA CAG ATT	774
	Ala Ile Gln Val Gln Asn Leu Leu Gln Leu Phe Asn Ile Pro Gln Ile	
	155 160 165	
35	GCT TAC TCT GCA ACC AGC ATG GAT TTG AGT GAC AAG ACT CTA TTC AAG	822
	Ala Tyr Ser Ala Thr Ser Met Asp Leu Ser Asp Lys Thr Leu Phe Lys	
	170 175 180 185	
40	TAC TTC ATG AGG GTT GTA CCT TCC GAT GCC CAG CAA GCT CGA GCC ATG	870
	Tyr Phe Met Arg Val Val Pro Ser Asp Ala Gln Gln Ala Arg Ala Met	
	190 195 200	
45	GTA GAC ATA GTG AAG AGA TAC AAC TGG ACT TAT GTC TCA GCT GTG CAC	918
	Val Asp Ile Val Lys Arg Tyr Asn Trp Thr Tyr Val Ser Ala Val His	
	205 210 215	
50	ACA GAA GGC AAC TAT GGA GAA AGT GGG ATG GAG GCT TTC AAA GAT ATG	966
	Thr Glu Gly Asn Tyr Gly Glu Ser Gly Met Glu Ala Phe Lys Asp Met	
	220 225 230	
55	TCA GCG AAG GAA GGG ATT TGC ATC GCC CAC TCT TAC AAA ATT TAC AGC	1014
	Ser Ala Lys Glu Gly Ile Cys Ile Ala His Ser Tyr Lys Ile Tyr Ser	
	235 240 245	
60	AAT GCT GGG GAA CAG AGC TTT GAC AAG CTG TTG AAA AAA CTC AGA AGT	1062
	Asn Ala Gly Glu Gln Ser Phe Asp Lys Leu Leu Lys Lys Leu Arg Ser	
	250 255 260 265	
65	CAT TTA CCT AAA GCC CGG GTG GTA GCT TGC TTC TGT GAA GGC ATG ACA	1110
	His Leu Pro Lys Ala Arg Val Val Ala Cys Phe Cys Glu Gly Met Thr	
	270 275 280	
70	GTT CGA GGT CTG CTC ATG GCC ATG AGA CGC TTA GGT CTA GCA GGG GAG	1158
	Val Arg Gly Leu Leu Met Ala Met Arg Arg Leu Gly Leu Ala Gly Glu	
	285 290 295	
75	TTT CTA CTT CTG GGC AGT GAT GGC TGG GGC GAC AGG TAT GAC GTG ACA	1206

	Phe	Leu	Leu	Leu	Gly	Ser	Asp	Gly	Trp	Ala	Asp	Arg	Tyr	Asp	Val	Thr	
			300					305					310				
5	GAT	GGA	TAT	CAG	CGA	GAA	GCT	GTC	GGT	GGA	ATT	ACA	ATC	AAG	CTT	CAG	1254
	Asp	Gly	Tyr	Gln	Arg	Glu	Ala	Val	Gly	Gly	Ile	Thr	Ile	Lys	Leu	Gln	
		315					320					325					
10	TCT	CCT	GAT	GTC	AAG	TGG	TTT	GAT	GAT	TAT	TAT	CTG	AAA	CTC	CGG	CCA	1302
	Ser	Pro	Asp	Val	Lys	Trp	Phe	Asp	Asp	Tyr	Tyr	Leu	Lys	Leu	Arg	Pro	
	330					335					340					345	
15	GAA	ACA	AAC	CTC	AGA	AAC	CCT	TGG	TTT	CAA	GAA	TTT	TGG	CAG	CAT	CGT	1350
	Glu	Thr	Asn	Leu	Arg	Asn	Pro	Trp	Phe	Gln	Glu	Phe	Trp	Gln	His	Arg	
					350					355					360		
20	TTT	CAG	TGC	CGG	CTG	GAA	GGG	TTT	GCA	CAG	GAG	AAC	AGC	AAG	TAC	AAC	1398
	Phe	Gln	Cys	Arg	Leu	Glu	Gly	Phe	Ala	Gln	Glu	Asn	Ser	Lys	Tyr	Asn	
				365					370					375			
25	AAG	ACT	TGC	AAC	AGT	TCT	CTG	ACT	CTG	AGA	ACA	CAT	CAT	GTT	CAA	GAT	1446
	Lys	Thr	Cys	Asn	Ser	Ser	Leu	Thr	Leu	Arg	Thr	His	His	Val	Gln	Asp	
			380					385					390				
30	TCC	AAA	ATG	GGA	TTT	GTG	ATA	AAT	GCA	ATC	TAT	TCT	ATG	GCT	TAT	GGA	1494
	Ser	Lys	Met	Gly	Phe	Val	Ile	Asn	Ala	Ile	Tyr	Ser	Met	Ala	Tyr	Gly	
		395					400					405					
35	CTC	CAC	AAT	ATG	CAG	ATG	TCC	CTG	TGT	CCA	GGC	TAT	GCA	GGC	CTC	TGT	1542
	Leu	His	Asn	Met	Gln	Met	Ser	Leu	Cys	Pro	Gly	Tyr	Ala	Gly	Leu	Cys	
	410					415					420					425	
40	GAT	GCG	ATG	AAG	CCA	ATT	GAT	GGA	CGG	AAA	CTT	TTG	GAT	TCC	CTG	ATG	1590
	Asp	Ala	Met	Lys	Pro	Ile	Asp	Gly	Arg	Lys	Leu	Leu	Asp	Ser	Leu	Met	
					430					435					440		
45	AAA	ACC	AAT	TTT	ACT	GGA	GTT	TCT	GGA	GAT	ATG	ATC	CTG	TTT	GAT	GAA	1638
	Lys	Thr	Asn	Phe	Thr	Gly	Val	Ser	Gly	Asp	Met	Ile	Leu	Phe	Asp	Glu	
				445					450					455			
50	AAT	GGA	GAC	TCT	CCA	GGA	AGG	TAT	GAA	ATA	ATG	AAT	TTC	AAG	GAA	ATG	1686
	Asn	Gly	Asp	Ser	Pro	Gly	Arg	Tyr	Glu	Ile	Met	Asn	Phe	Lys	Glu	Met	
			460					465					470				
55	GGA	AAA	GAT	TAT	TTT	GAT	TAC	ATC	AAT	GTT	GGA	AGT	TGG	GAC	AAT	GGG	1734
	Gly	Lys	Asp	Tyr	Phe	Asp	Tyr	Ile	Asn	Val	Gly	Ser	Trp	Asp	Asn	Gly	
		475					480					485					
60	GAA	TTA	AAA	ATG	GAT	GAT	GAC	GAA	GTG	TGG	TCC	AAG	AAA	AAT	AAC	ATC	1782
	Glu	Leu	Lys	Met	Asp	Asp	Asp	Glu	Val	Trp	Ser	Lys	Lys	Asn	Asn	Ile	
	490					495					500					505	
65	ATC	AGA	TCT	GTG	TGC	AGT	GAA	CCA	TGT	GAG	AAA	GGC	CAA	ATA	AAG	GTG	1830
	Ile	Arg	Ser	Val	Cys	Ser	Glu	Pro	Cys	Glu	Lys	Gly	Gln	Ile	Lys	Val	
					510					515					520		
70	ATC	CGG	AAG	GGA	GAA	GTC	AGC	TGT	TGT	TGG	ACC	TGC	ACA	CCT	TGT	AAA	1878
	Ile	Arg	Lys	Gly	Glu	Val	Ser	Cys	Cys	Trp	Thr	Cys	Thr	Pro	Cys	Lys	
				525					530					535			
75	GAG	AAT	GAG	TAT	GTG	TTT	GAT	GAG	TAC	ACC	TGC	AAG	GCA	TGC	CAA	CTG	1926
	Glu	Asn	Glu	Tyr	Val	Phe	Asp	Glu	Tyr	Thr	Cys	Lys	Ala	Cys	Gln	Leu	

	540	545	550	
5	GGC TCC TGG CCC ACT GAC GAC CTG ACA GGC TGT GAC TTG ATC CCA GTC Gly Ser Trp Pro Thr Asp Asp Leu Thr Gly Cys Asp Leu Ile Pro Val 555 560 565	1974		
10	CAG TAT CTT CGC TGG GGT GAC CCT GAG CCG ATT GCA GCT GTG GTG TTT Gln Tyr Leu Arg Trp Gly Asp Pro Glu Pro Ile Ala Ala Val Val Phe 570 575 580 585	2022		
15	GCC TGC CTC GGT CTG CTA GCC ACC TTA TTC GTT ACT GTA ATC TTC ATC Ala Cys Leu Gly Leu Leu Ala Thr Leu Phe Val Thr Val Ile Phe Ile 590 595 600	2070		
20	ATT TAT CGG GAC ACT CCG GTG GTC AAG TCC TCC AGC AGG GAG CTC TGC Ile Tyr Arg Asp Thr Pro Val Val Lys Ser Ser Ser Arg Glu Leu Cys 605 610 615	2118		
25	TAC ATT ATC CTT GCT GGC ATC TGC CTG GGA TAC TTA TGT ACC TTT TGC Tyr Ile Ile Leu Ala Gly Ile Cys Leu Gly Tyr Leu Cys Thr Phe Cys 620 625 630	2166		
30	CTC ATT GCA AAG CCC AAG CAG ATT TAC TGC TAT CTT CAG AGA ATT GGC Leu Ile Ala Lys Pro Lys Gln Ile Tyr Cys Tyr Leu Gln Arg Ile Gly 635 640 645	2214		
35	ATT GGA CTC TCT CCA GCC ATG AGC TAC TCA GCC CTT GTA ACC AAG ACC Ile Gly Leu Ser Pro Ala Met Ser Tyr Ser Ala Leu Val Thr Lys Thr 650 655 660 665	2262		
40	AAC CGT ATT GCA AGG ATT CTA GCT GGA AGC AAG AAG AAA ATC TGT ACC Asn Arg Ile Ala Arg Ile Leu Ala Gly Ser Lys Lys Lys Ile Cys Thr 670 675 680	2310		
45	AAG AAG CCC AGA TTC ATG AGC GCC TGT GCT CAG TTA GTG ATC GCT TTC Lys Lys Pro Arg Phe Met Ser Ala Cys Ala Gln Leu Val Ile Ala Phe 685 690 695	2358		
50	ATT CTC ATC TGT ATT CAG CTG GGC ATT ATC GTG GCC CTC TTT ATC ATG Ile Leu Ile Cys Ile Gln Leu Gly Ile Ile Val Ala Leu Phe Ile Met 700 705 710	2406		
55	GAG CCT CCG GAT ATA ATG CAT GAC TAT CCA AGC ATC CGA GAA GTC TAC Glu Pro Pro Asp Ile Met His Asp Tyr Pro Ser Ile Arg Glu Val Tyr 715 720 725	2454		
60	TTG ATT TGT AAC ACC ACC AAC CTA GGG GTT GTT ACT CCT CTT GGA TAC Leu Ile Cys Asn Thr Thr Asn Leu Gly Val Val Thr Pro Leu Gly Tyr 730 735 740 745	2502		
65	AAT GGA TTA TTG ATT TTG AGT TGC ACG TTC TAT GCG TTT AAG ACC AGA Asn Gly Leu Leu Ile Leu Ser Cys Thr Phe Tyr Ala Phe Lys Thr Arg 750 755 760	2550		
70	AAT GTT CCA GCC AAC TTT AAT GAG GCC AAA TAT ATT GCT TTC ACC ATG Asn Val Pro Ala Asn Phe Asn Glu Ala Lys Tyr Ile Ala Phe Thr Met 765 770 775	2598		
75	TAC ACA ACC TGC ATC ATA TGG CTG GCC TTC GTG CCT ATC TAC TTT GGC Tyr Thr Thr Cys Ile Ile Trp Leu Ala Phe Val Pro Ile Tyr Phe Gly 780 785 790	2646		

5	AGC AAC TAC AAA ATC ATC ACC ATG TGT TTT TCA GTC AGC CTC AGT GCC 2694 Ser Asn Tyr Lys Ile Ile Thr Met Cys Phe Ser Val Ser Leu Ser Ala 795 800 805
	ACA GTG GCC CTG GGT TGC ATG TTT GTC CCG AAG GTG TAC ATC ATC CTA 2742 Thr Val Ala Leu Gly Cys Met Phe Val Pro Lys Val Tyr Ile Ile Leu 810 815 820 825
10	GCC AAA CCG GAG AGA AAT GTG CGC AGC GGC TTC ACA ACC TCT ACA GTG 2790 Ala Lys Pro Glu Arg Asn Val Arg Ser Ala Phe Thr Thr Ser Thr Val 830 835 840
15	GTA CGC ATG CAT GTA GGA GAC GGC AAA TCA TCG TCC GCT GCC AGC AGA 2838 Val Arg Met His Val Gly Asp Gly Lys Ser Ser Ser Ala Ala Ser Arg 845 850 855
20	TCC AGC AGC CTA GTC AAC CTG TGG AAG AGG AGG GGC TCC TCT GGG GAA 2886 Ser Ser Ser Leu Val Asn Leu Trp Lys Arg Arg Gly Ser Ser Gly Glu 860 865 870
25	ACC CTA AGC TCC AAC GGA AAA TCT GTG ACT TGG GCC CAG AAT GAG AAG 2934 Thr Leu Ser Ser Asn Gly Lys Ser Val Thr Trp Ala Gln Asn Glu Lys 875 880 885
30	AGT ACC CCG GGG CAA CAT TTG TGG CAG CGA CTG TCT GTC CAC ATC AAC 2982 Ser Thr Arg Gly Gln His Leu Trp Gln Arg Leu Ser Val His Ile Asn 890 895 900 905
35	AAG AAG GAG AAC CCC AAC CAA ACG GCG GTC ATC AAA CCC TTT CCC AAG 3030 Lys Lys Glu Asn Pro Asn Gln Thr Ala Val Ile Lys Pro Phe Pro Lys 910 915 920
40	AGC ACA GAG AAC CGG GGG CCT GGT GCA GCG GCA GGT GGT GGC TCG GGT 3078 Ser Thr Glu Asn Arg Gly Pro Gly Ala Ala Ala Gly Gly Gly Ser Gly 925 930 935
45	CCC GGT GTA GCT GGT GCT GGT AAC GCA GGA TGC ACA GCA ACA GGT GGC 3126 Pro Gly Val Ala Gly Ala Gly Asn Ala Gly Cys Thr Ala Thr Gly Gly 940 945 950
50	CCG GAG CCA CCG GAC GCC GGT CCC AAA GCG CTT TAT GAT GTG GCG GAG 3174 Pro Glu Pro Pro Asp Ala Gly Pro Lys Ala Leu Tyr Asp Val Ala Glu 955 960 965
55	GCA GAG GAG AGC TTC CCC GCG GCT GCC AGG CCG CGC TCG CCA TCG CCC 3222 Ala Glu Glu Ser Phe Pro Ala Ala Ala Arg Pro Arg Ser Pro Ser Pro 970 975 980 985
60	ATC AGC ACG TTG AGC CAC CTC GCA GGC TCG GCG GGC CGC ACA GAT GAT 3270 Ile Ser Thr Leu Ser His Leu Ala Gly Ser Ala Gly Arg Thr Asp Asp 990 995 1000
	GAT GCG CCG TCG CTG CAC TCT GAG ACA GCT GCT CGC AGC AGC TCG TCC 3318 Asp Ala Pro Ser Leu His Ser Glu Thr Ala Ala Arg Ser Ser Ser Ser 1005 1010 1015
	CAG GGC TCG CTC ATG GAG CAG ATC AGC AGC GTA GTG ACG CGC TTC ACA 3366 Gln Gly Ser Leu Met Glu Gln Ile Ser Ser Val Val Thr Arg Phe Thr 1020 1025 1030

	CCC AAC ATC TCC GAG CTC AAC TCC ATG ATG TTG TCC ACC GCG GCG ACG Ala Asn Ile Ser Glu Leu Asn Ser Met Met Leu Ser Thr Ala Ala Thr 1035 1040 1045	3414
5	CCG GCG CCC CCT GGT ACT CCA ATC TGC TCC TCC TAC CTG ATC CCC AAA Pro Gly Pro Pro Gly Thr Pro Ile Cys Ser Ser Tyr Leu Ile Pro Lys 1050 1055 1060 1065	3462
10	GAG ATT CAG CTG CCC ACG ACC ATG ACG ACC TTC GCA GAG ATC CAG CCG Glu Ile Gln Leu Pro Thr Thr Met Thr Thr Phe Ala Glu Ile Gln Pro 1070 1075 1080	3510
15	CTG CCG GCG ATC GAG GTG ACC GGA GGA GCT CAG GGG GCG ACA GGC GTA Leu Pro Ala Ile Glu Val Thr Gly Gly Ala Gln Gly Ala Thr Gly Val 1085 1090 1095	3558
20	TCA CCT GCG CAG GAG ACG CCC ACA GGA GCT GAA TCC GCC CCG GGC AAA Ser Pro Ala Gln Glu Thr Pro Thr Gly Ala Glu Ser Ala Pro Gly Lys 1100 1105 1110	3606
25	CCA GAT CTG GAG GAG CTT GTG GCC CTC ACT CCA CCA TCG CCC TTC AGG Pro Asp Leu Glu Glu Leu Val Ala Leu Thr Pro Pro Ser Pro Phe Arg 1115 1120 1125	3654
30	GAC TCG GTG GAC TCG GGG AGC ACC ACC CCA AAC TCT CCA GTC TCA GAA Asp Ser Val Asp Ser Gly Ser Thr Thr Pro Asn Ser Pro Val Ser Glu 1130 1135 1140 1145	3702
35	TCG GCC CTC TGC ATC CCA TCC TCT CCC AAA TAT GAC ACT CTC ATC ATC Ser Ala Leu Cys Ile Pro Ser Ser Pro Lys Tyr Asp Thr Leu Ile Ile 1150 1155 1160	3750
40	AGA GAT TAC ACG CAG AGT TCT TCA TCG TTG TGAGCCACTG GAAACTTCCC TGA Arg Asp Tyr Thr Gln Ser Ser Ser Ser Leu 1165 1170	3803
45	GATAGGCCTG TGCAGCAGAC CTCGGTGTTC CAGATGGGCG CAGCGACACC CATAGTCACC CCAACAGAGA ACCCGAGGTG AAAAATGCCA AGGACACACA CGGTGGAGAC ATGAG	3863 3918
50	(2) INFORMATION FOR SEQ ID NO:6:	
55	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 1171 amino acids	
	(B) TYPE: amino acid	
	(C) STRANDEDNESS: single	
	(D) TOPOLOGY: linear	
60	(ii) MOLECULE TYPE: protein	
	(v) FRAGMENT TYPE: internal	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:	
	Met Val Leu Leu Leu Ile Leu Ser Val Leu Leu Leu Lys Glu Asp Val 1 5 10 15	
55	Arg Gly Ser Ala Gln Ser Ser Glu Arg Arg Val Val Ala His Met Pro 20 25 30	
	Gly Asp Ile Ile Ile Gly Ala Leu Phe Ser Val His His Gln Pro Thr 35 40 45	
60	Val Asp Lys Val His Glu Arg Lys Cys Gly Ala Val Arg Glu Gln Tyr 50 55 60	
	Gly Ile Gln Arg Val Glu Ala Met Leu His Thr Leu Glu Arg Ile Asn	

	65					70				75					80	
	Ser	Asp	Pro	Thr	Leu	Leu	Pro	Asn	Ile	Thr	Leu	Gly	Cys	Glu	Ile	Arg
					85					90					95	
5	Asp	Ser	Cys	Trp	His	Ser	Ala	Val	Ala	Leu	Glu	Gln	Ser	Ile	Glu	Phe
				100					105					110		
	Ile	Arg	Asp	Ser	Leu	Ile	Ser	Ser	Glu	Glu	Glu	Glu	Gly	Leu	Val	Arg
			115					120					125			
	Cys	Val	Asp	Gly	Ser	Ser	Ser	Phe	Arg	Ser	Lys	Lys	Pro	Ile	Val	Gly
			130					135				140				
10	Val	Ile	Gly	Pro	Gly	Ser	Ser	Ser	Val	Ala	Ile	Gln	Val	Gln	Asn	Leu
				145				150			155					160
	Leu	Gln	Leu	Phe	Asn	Ile	Pro	Gln	Ile	Ala	Tyr	Ser	Ala	Thr	Ser	Met
					165				170						175	
15	Asp	Leu	Ser	Asp	Lys	Thr	Leu	Phe	Lys	Tyr	Phe	Met	Arg	Val	Val	Pro
				180					185					190		
	Ser	Asp	Ala	Gln	Gln	Ala	Arg	Ala	Met	Val	Asp	Ile	Val	Lys	Arg	Tyr
			195					200					205			
	Asn	Trp	Thr	Tyr	Val	Ser	Ala	Val	His	Thr	Glu	Gly	Asn	Tyr	Gly	Glu
			210				215					220				
20	Ser	Gly	Met	Glu	Ala	Phe	Lys	Asp	Met	Ser	Ala	Lys	Glu	Gly	Ile	Cys
						230					235					240
	Ile	Ala	His	Ser	Tyr	Lys	Ile	Tyr	Ser	Asn	Ala	Gly	Glu	Gln	Ser	Phe
					245					250					255	
25	Asp	Lys	Leu	Leu	Lys	Lys	Leu	Arg	Ser	His	Leu	Pro	Lys	Ala	Arg	Val
				260					265					270		
	Val	Ala	Cys	Phe	Cys	Glu	Gly	Met	Thr	Val	Arg	Gly	Leu	Leu	Met	Ala
			275						280					285		
	Met	Arg	Arg	Leu	Gly	Leu	Ala	Gly	Glu	Phe	Leu	Leu	Leu	Gly	Ser	Asp
			290				295					300				
30	Gly	Trp	Ala	Asp	Arg	Tyr	Asp	Val	Thr	Asp	Gly	Tyr	Gln	Arg	Glu	Ala
						310					315					320
	Val	Gly	Gly	Ile	Thr	Ile	Lys	Leu	Gln	Ser	Pro	Asp	Val	Lys	Trp	Phe
					325					330					335	
35	Asp	Asp	Tyr	Tyr	Leu	Lys	Leu	Arg	Pro	Glu	Thr	Asn	Leu	Arg	Asn	Pro
				340					345					350		
	Trp	Phe	Gln	Glu	Phe	Trp	Gln	His	Arg	Phe	Gln	Cys	Arg	Leu	Glu	Gly
				355					360					365		
	Phe	Ala	Gln	Glu	Asn	Ser	Lys	Tyr	Asn	Lys	Thr	Cys	Asn	Ser	Ser	Leu
			370				375					380				
40	Thr	Leu	Arg	Thr	His	His	Val	Gln	Asp	Ser	Lys	Met	Gly	Phe	Val	Ile
						390					395					400
	Asn	Ala	Ile	Tyr	Ser	Met	Ala	Tyr	Gly	Leu	His	Asn	Met	Gln	Met	Ser
					405					410					415	
45	Leu	Cys	Pro	Gly	Tyr	Ala	Gly	Leu	Cys	Asp	Ala	Met	Lys	Pro	Ile	Asp
				420					425					430		
	Gly	Arg	Lys	Leu	Leu	Asp	Ser	Leu	Met	Lys	Thr	Asn	Phe	Thr	Gly	Val
			435						440					445		
	Ser	Gly	Asp	Met	Ile	Leu	Phe	Asp	Glu	Asn	Gly	Asp	Ser	Pro	Gly	Arg
			450				455					460				
50	Tyr	Glu	Ile	Met	Asn	Phe	Lys	Glu	Met	Gly	Lys	Asp	Tyr	Phe	Asp	Tyr
						470					475					480
	Ile	Asn	Val	Gly	Ser	Trp	Asp	Asn	Gly	Glu	Leu	Lys	Met	Asp	Asp	Asp
					485					490					495	
55	Glu	Val	Trp	Ser	Lys	Lys	Asn	Asn	Ile	Ile	Arg	Ser	Val	Cys	Ser	Glu
				500					505					510		
	Pro	Cys	Glu	Lys	Gly	Gln	Ile	Lys	Val	Ile	Arg	Lys	Gly	Glu	Val	Ser
			515						520					525		
	Cys	Cys	Trp	Thr	Cys	Thr	Pro	Cys	Lys	Glu	Asn	Glu	Tyr	Val	Phe	Asp
			530				535					540				
60	Glu	Tyr	Thr	Cys	Lys	Ala	Cys	Gln	Leu	Gly	Ser	Trp	Pro	Thr	Asp	Asp
						550					555					560

	Leu	Thr	Gly	Cys	Asp	Leu	Ile	Pro	Val	Gln	Tyr	Leu	Arg	Trp	Gly	Asp
					565					570					575	
	Pro	Glu	Pro	Ile	Ala	Ala	Val	Val	Phe	Ala	Cys	Leu	Gly	Leu	Leu	Ala
					580					585					590	
5	Thr	Leu	Phe	Val	Thr	Val	Ile	Phe	Ile	Ile	Tyr	Arg	Asp	Thr	Pro	Val
			595					600					605			
	Val	Lys	Ser	Ser	Ser	Arg	Glu	Leu	Cys	Tyr	Ile	Ile	Leu	Ala	Gly	Ile
		610					615					620				
10	Cys	Leu	Gly	Tyr	Leu	Cys	Thr	Phe	Cys	Leu	Ile	Ala	Lys	Pro	Lys	Gln
		625				630						635				640
	Ile	Tyr	Cys	Tyr	Leu	Gln	Arg	Ile	Gly	Ile	Gly	Leu	Ser	Pro	Ala	Met
					645					650					655	
	Ser	Tyr	Ser	Ala	Leu	Val	Thr	Lys	Thr	Asn	Arg	Ile	Ala	Arg	Ile	Leu
				660					665					670		
15	Ala	Gly	Ser	Lys	Lys	Lys	Ile	Cys	Thr	Lys	Lys	Pro	Arg	Phe	Met	Ser
			675					680						685		
	Ala	Cys	Ala	Gln	Leu	Val	Ile	Ala	Phe	Ile	Leu	Ile	Cys	Ile	Gln	Leu
			690					695					700			
20	Gly	Ile	Ile	Val	Ala	Leu	Phe	Ile	Met	Glu	Pro	Pro	Asp	Ile	Met	His
		705				710						715				720
	Asp	Tyr	Pro	Ser	Ile	Arg	Glu	Val	Tyr	Leu	Ile	Cys	Asn	Thr	Thr	Asn
					725					730					735	
	Leu	Gly	Val	Val	Thr	Pro	Leu	Gly	Tyr	Asn	Gly	Leu	Leu	Ile	Leu	Ser
				740					745					750		
25	Cys	Thr	Phe	Tyr	Ala	Phe	Lys	Thr	Arg	Asn	Val	Pro	Ala	Asn	Phe	Asn
			755					760						765		
	Glu	Ala	Lys	Tyr	Ile	Ala	Phe	Thr	Met	Tyr	Thr	Thr	Cys	Ile	Ile	Trp
		770					775						780			
30	Leu	Ala	Phe	Val	Pro	Ile	Tyr	Phe	Gly	Ser	Asn	Tyr	Lys	Ile	Ile	Thr
		785				790						795				800
	Met	Cys	Phe	Ser	Val	Ser	Leu	Ser	Ala	Thr	Val	Ala	Leu	Gly	Cys	Met
					805					810					815	
	Phe	Val	Pro	Lys	Val	Tyr	Ile	Ile	Leu	Ala	Lys	Pro	Glu	Arg	Asn	Val
				820					825					830		
35	Arg	Ser	Ala	Phe	Thr	Thr	Ser	Thr	Val	Val	Arg	Met	His	Val	Gly	Asp
			835					840					845			
	Gly	Lys	Ser	Ser	Ser	Ala	Ala	Ser	Arg	Ser	Ser	Ser	Leu	Val	Asn	Leu
		850					855					860				
40	Trp	Lys	Arg	Arg	Gly	Ser	Ser	Gly	Glu	Thr	Leu	Ser	Ser	Asn	Gly	Lys
		865				870					875				880	
	Ser	Val	Thr	Trp	Ala	Gln	Asn	Glu	Lys	Ser	Thr	Arg	Gly	Gln	His	Leu
					885					890					895	
	Trp	Gln	Arg	Leu	Ser	Val	His	Ile	Asn	Lys	Lys	Glu	Asn	Pro	Asn	Gln
				900						905				910		
45	Thr	Ala	Val	Ile	Lys	Pro	Phe	Pro	Lys	Ser	Thr	Glu	Asn	Arg	Gly	Pro
			915					920					925			
	Gly	Ala	Ala	Ala	Gly	Gly	Gly	Ser	Gly	Pro	Gly	Val	Ala	Gly	Ala	Gly
		930					935					940				
50	Asn	Ala	Gly	Cys	Thr	Ala	Thr	Gly	Gly	Pro	Glu	Pro	Pro	Asp	Ala	Gly
		945				950					955				960	
	Pro	Lys	Ala	Leu	Tyr	Asp	Val	Ala	Glu	Ala	Glu	Glu	Ser	Phe	Pro	Ala
					965					970					975	
	Ala	Ala	Arg	Pro	Arg	Ser	Pro	Ser	Pro	Ile	Ser	Thr	Leu	Ser	His	Leu
				980					985					990		
55	Ala	Gly	Ser	Ala	Gly	Arg	Thr	Asp	Asp	Asp	Ala	Pro	Ser	Leu	His	Ser
			995					1000					1005			
	Glu	Thr	Ala	Ala	Arg	Ser	Ser	Ser	Ser	Gln	Gly	Ser	Leu	Met	Glu	Gln
		1010					1015					1020				
60	Ile	Ser	Ser	Val	Val	Thr	Arg	Phe	Thr	Ala	Asn	Ile	Ser	Glu	Leu	Asn
		025					1030				1035				1040	
	Ser	Met	Met	Leu	Ser	Thr	Ala	Ala	Thr	Pro	Gly	Pro	Pro	Gly	Thr	Pro

1045 1050 1055
 Ile Cys Ser Ser Tyr Leu Ile Pro Lys Glu Ile Gln Leu Pro Thr Thr
 1060 1065 1070
 Met Thr Thr Phe Ala Glu Ile Gln Pro Leu Pro Ala Ile Glu Val Thr
 1075 1080 1085
 Gly Gly Ala Gln Gly Ala Thr Gly Val Ser Pro Ala Gln Glu Thr Pro
 1090 1095 1100
 Thr Gly Ala Glu Ser Ala Pro Gly Lys Pro Asp Leu Glu Glu Leu Val
 1105 1110 1115 1120
 Ala Leu Thr Pro Pro Ser Pro Phe Arg Asp Ser Val Asp Ser Gly Ser
 1125 1130 1135
 Thr Thr Pro Asn Ser Pro Val Ser Glu Ser Ala Leu Cys Ile Pro Ser
 1140 1145 1150
 Ser Pro Lys Tyr Asp Thr Leu Ile Ile Arg Asp Tyr Thr Gln Ser Ser
 1155 1160 1165
 Ser Ser Leu
 1170

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1212 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Val Leu Leu Leu Ile Leu Ser Val Leu Leu Leu Lys Glu Asp Val
 1 5 10 15
 Arg Gly Ser Ala Gln Ser Ser Glu Arg Arg Val Val Ala His Met Pro
 20 25 30
 Gly Asp Ile Ile Ile Gly Ala Leu Phe Ser Val His His Gln Pro Thr
 35 40 45
 Val Asp Lys Val His Glu Arg Lys Cys Gly Ala Val Arg Glu Gln Tyr
 50 55 60
 Gly Ile Gln Arg Val Glu Ala Met Leu His Thr Leu Glu Arg Ile Asn
 65 70 75 80
 Ser Asp Pro Thr Leu Leu Pro Asn Ile Thr Leu Gly Cys Glu Ile Arg
 85 90 95
 Asp Ser Cys Trp His Ser Ala Val Ala Leu Glu Gln Ser Ile Glu Phe
 100 105 110
 Ile Arg Asp Ser Leu Ile Ser Ser Glu Glu Glu Gly Leu Val Arg
 115 120 125
 Cys Val Asp Gly Ser Ser Ser Ser Phe Arg Ser Lys Lys Pro Ile Val
 130 135 140
 Gly Val Ile Gly Pro Gly Ser Ser Ser Val Ala Ile Gln Val Gln Asn
 145 150 155 160
 Leu Leu Gln Leu Phe Asn Ile Pro Gln Ile Ala Tyr Ser Ala Thr Ser
 165 170 175
 Met Asp Leu Ser Asp Lys Thr Leu Phe Lys Tyr Phe Met Arg Val Val
 180 185 190
 Pro Ser Asp Ala Gln Gln Ala Arg Ala Met Val Asp Ile Val Lys Arg
 195 200 205
 Tyr Asn Trp Thr Tyr Val Ser Ala Val His Thr Glu Gly Asn Tyr Gly
 210 215 220
 Glu Ser Gly Met Glu Ala Phe Lys Asp Met Ser Ala Lys Glu Gly Ile
 225 230 235 240
 Cys Ile Ala His Ser Tyr Lys Ile Tyr Ser Asn Ala Gly Glu Gln Ser
 245 250 255
 Phe Asp Lys Leu Leu Lys Lys Leu Thr Ser His Leu Pro Lys Ala Arg

						260											265																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
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	Ser	Cys	Thr	Phe	Tyr	Ala	Phe	Lys	Thr	Arg	Asn	Val	Pro	Ala	Asn	Phe
			755					760					765			
	Asn	Glu	Ala	Lys	Tyr	Ile	Ala	Phe	Thr	Met	Tyr	Thr	Thr	Cys	Ile	Ile
		770					775					780				
5	Trp	Leu	Ala	Phe	Val	Pro	Ile	Tyr	Phe	Gly	Ser	Asn	Tyr	Lys	Ile	Ile
	785					790					795				800	
	Thr	Met	Cys	Phe	Ser	Val	Ser	Leu	Ser	Ala	Thr	Val	Ala	Leu	Gly	Cys
				805						810					815	
	Met	Phe	Val	Pro	Lys	Val	Tyr	Ile	Ile	Leu	Ala	Lys	Pro	Glu	Arg	Asn
10				820						825				830		
	Val	Arg	Ser	Ala	Phe	Thr	Thr	Ser	Thr	Val	Val	Arg	Met	His	Val	Gly
				835				840					845			
	Asp	Gly	Lys	Ser	Ser	Ser	Ala	Ala	Ser	Arg	Ser	Ser	Ser	Leu	Val	Asn
		850					855					860				
15	Leu	Trp	Lys	Arg	Arg	Gly	Ser	Ser	Gly	Glu	Thr	Leu	Arg	Tyr	Lys	Asp
	865					870					875				880	
	Arg	Arg	Leu	Ala	Gln	His	Lys	Ser	Glu	Ile	Glu	Cys	Phe	Thr	Pro	Lys
				885						890					895	
	Gly	Ser	Met	Gly	Asn	Gly	Gly	Arg	Ala	Thr	Met	Ser	Ser	Ser	Asn	Gly
20				900					905					910		
	Lys	Ser	Val	Thr	Trp	Ala	Gln	Asn	Glu	Lys	Ser	Ser	Arg	Gly	Gln	His
			915					920					925			
	Leu	Trp	Gln	Arg	Leu	Ser	Ile	His	Ile	Asn	Lys	Lys	Glu	Asn	Pro	Asn
		930					935					940				
25	Gln	Thr	Ala	Val	Ile	Lys	Pro	Phe	Pro	Lys	Ser	Thr	Glu	Ser	Arg	Gly
		945				950					955				960	
	Leu	Gly	Ala	Gly	Ala	Gly	Ala	Gly	Gly	Ser	Ala	Gly	Gly	Val	Gly	Ala
				965						970					975	
	Thr	Gly	Gly	Ala	Gly	Cys	Ala	Gly	Ala	Gly	Pro	Gly	Gly	Pro	Glu	Ser
30				980					985					990		
	Pro	Asp	Ala	Gly	Pro	Lys	Ala	Leu	Tyr	Asp	Val	Ala	Glu	Ala	Glu	Glu
			995				1000					1005				
	His	Phe	Pro	Ala	Pro	Ala	Arg	Pro	Arg	Ser	Pro	Ser	Pro	Ile	Ser	Thr
		1010				1015					1020					
35	Leu	Ser	His	Arg	Ala	Gly	Ser	Ala	Ser	Arg	Thr	Asp	Asp	Asp	Val	Pro
		025			1030					1035					1040	
	Ser	Leu	His	Ser	Glu	Pro	Val	Ala	Arg	Ser	Ser	Ser	Ser	Gln	Gly	Ser
				1045					1050					1055		
	Leu	Met	Glu	Gln	Ile	Ser	Ser	Val	Val	Thr	Arg	Phe	Thr	Ala	Asn	Ile
40			1060					1065						1070		
	Ser	Glu	Leu	Asn	Ser	Met	Met	Leu	Ser	Thr	Ala	Ala	Pro	Ser	Pro	Gly
		1075				1080					1085					
	Val	Gly	Ala	Pro	Leu	Cys	Ser	Ser	Tyr	Leu	Ile	Pro	Lys	Glu	Ile	Gln
		1090			1095						1100					
45	Leu	Pro	Thr	Thr	Met	Thr	Thr	Phe	Ala	Glu	Ile	Gln	Pro	Leu	Pro	Ala
		105			1110					1115					1120	
	Ile	Glu	Val	Thr	Gly	Gly	Ala	Gln	Pro	Ala	Ala	Gly	Ala	Gln	Ala	Ala
				1125					1130					1135		
	Gly	Asp	Ala	Ala	Arg	Glu	Ser	Pro	Ala	Ala	Gly	Pro	Glu	Ala	Ala	Ala
50			1140					1145					1150			
	Ala	Lys	Pro	Asp	Leu	Glu	Glu	Leu	Val	Ala	Leu	Thr	Pro	Pro	Ser	Pro
		1155				1160					1165					
	Phe	Arg	Asp	Ser	Val	Asp	Ser	Gly	Ser	Thr	Thr	Pro	Asn	Ser	Pro	Val
		1170				1175					1180					
55	Ser	Glu	Ser	Ala	Leu	Cys	Ile	Pro	Ser	Ser	Pro	Lys	Tyr	Asp	Thr	Leu
		185			1190					1195					1200	
	Ile	Ile	Arg	Asp	Tyr	Thr	Gln	Ser	Ser	Ser	Ser	Leu				
				1205						1210						

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT.US99/02033 (22) International Filing Date: 29 January 1999 (29.01.99) (30) Priority Data: 60/073,190 30 January 1998 (30.01.98) US (71) Applicant (for all designated States except US): UNIVER- SITY TECHNOLOGY CORPORATION [US/US]; Suite 250, 3101 Iris Avenue, Boulder, CO 80301 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): JOHNSON, Thomas, E. [US/US]; 1121 West Enclave Circle, Louisville, CO 80027 (US). SIKELA, James, M. [US/US]; 6046 S. Kingston Circle, Englewood, CO 80111 (US). SIMPSON, Victoria, J. [US/US]; 1121 W. Enclave Circle, Englewood, CO 80027 (US). RIKKE, Brad, A. [US/US]; 750 Hartford, Boulder, CO 80303 (US). (74) Agents: POLIZZZI, Catherine, M. et al.; Morrison & Foerster LLP, 755 Page Mill Road, Palo Alto, CA 94304-1018 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims</i> <i>and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 23 September 1999 (23.09.99)	
(54) Title: POLYNUCLEOTIDE AND POLYPEPTIDE SEQUENCES ASSOCIATED WITH CNS DEPRESSANT SENSITIVITY AND METHODS OF USE THEREOF			
(57) Abstract The invention provides polynucleotide and polypeptide sequences associated with CNS depressant sensitivity, and methods for identifying and classifying candidate CNS depressants as well as methods to identify agents which may modulate CNS depressant action. The invention also provides mouse mGluR5 polynucleotide and polypeptide sequences.			

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 99/02033

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/705 G01N33/68 C07K16/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 29449 A (SALK INST BIOTECH IND ;DAGGETT LORRIE (US); ELLIS STEVEN B (US); L) 22 December 1994 cited in the application see claim 26; example 1 ---	10-19
X	SOMMER, B. ET AL.: "RNA editing in brain controls a determinant of ion flow in glutamate-gated channels." CELL, vol. 67, 4 October 1991, pages 11-9, XP002108292 see page 16, right-hand column, paragraph 5 --- -/--	20-35



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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"P" document published prior to the international filing date but later than the priority date claimed

T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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Y* document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

S* document member of the same patent family

Date of the actual completion of the international search

6 July 1999

Date of mailing of the international search report

27/07/1999

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Authorized officer

Smalt, R

INTERNATIONAL SEARCH REPORT

Int'l. Patent Application No.

PCT/US 99/02033

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>MINAKAMI, R. ET AL.: "The expression of two splice variants of metabotropic glutamate receptor subtype 5 in the rat brain and neural cells during development." JOURNAL OF NEUROCHEMISTRY, vol. 65, no. 4, October 1995, pages 1536-42, XP002108290 cited in the application see the whole document</p>	20-35
X	<p>DORRI, F. ET AL.: "Down-regulation of mGluR5 by antisense deoxynucleotides alters pharmacological responses to application of ACPD in the rat hippocampus." EXPERIMENTAL NEUROLOGY, vol. 147, no. 1, September 1997, pages 48-54, XP002108293 see abstract see page 50, left-hand column, paragraph 2</p>	37, 38
Y	<p>MINAMI, K. ET AL.: "Effects of ethanol and anesthetics on type 1 and 5 metabotropic glutamate receptors expressed in <i>Xenopus laevis</i> oocytes." MOLECULAR PHARMACOLOGY, vol. 53, no. 1, 1 January 1998, pages 148-156, XP002108294 see abstract</p>	1-9
Y	<p>SIMPSON, V.J. ET AL.: "Mapping a major QTL for propofol neurosensitivity in F2 mice from an LS by SS cross." ANESTHESIOLOGY, vol. 65, no. 3a, September 1996, page a627 XP002108295 see the whole document</p>	1-9
A	<p>SCHEDL A ET AL: "A YEAST ARTIFICIAL CHROMOSOME COVERING THE TYROSINASE GENE CONFERS COPY NUMBER-DEPENDENT EXPRESSION IN TRANSGENIC MICE" NATURE, vol. 362, no. 6417, 18 March 1993, pages 258-261, XP000563891 cited in the application see the whole document</p>	1-9

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INTERNATIONAL SEARCH REPORT

In International Application No.
PCT/US 99/02033

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>PROCTER, M. ET AL.: "Possible role of GluR5 glutamate receptors in spinal nociceptive processing in the anaesthetized rat." JOURNAL OF PHYSIOLOGY, vol. 504P, November 1997, pages 101P-102P, XP002108296 see the whole document ----</p>	
A	<p>ABE T ET AL: "MOLECULAR CHARACTERIZATION OF A NOVEL METABOTROPIC GLUTAMATE RECEPTOR MGLUR5 COUPLED TO INOSITOL PHOSPHATE/CA2+ SIGNAL TRANSDUCTION" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 267, no. 19, 5 July 1992, pages 13361-13368, XP000605387 cited in the application see the whole document ----</p>	
T	<p>SIMPSON, V.J. ET AL.: "Ketamine exerts differential hypnotic effects in long sleep and short sleep mice." ANESTHESIOLOGY, vol. 89, no. 3a, September 1998, page A128 XP002108299 see the whole document -----</p>	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 99/02033

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-9, 20-38 completely, and 10,11,13,15,16 partially
Mouse mGluR5 and its uses in screening and characterising CNS depressants.
2. Claims : 18 completely, and 10-13,15-17 partially
The use of human mGluR5 in screening and characterising CNS depressants.
3. Claims; 10,11,14-16 partially
The use of mouse mGluR1 in screening and characterising CNS depressants.
4. Claims: 19 completely, and 10-12,14-17 partially
The use of human mGluR1 in screening and characterising CNS depressants
5. Claims: 10-17 partially
The use of metabotropic glutamate receptors, other than human mGluR1, human mGluR5, mouse mGluR1, and mouse mGluR5, in screening and characterising CNS depressants.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/02033

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